JOURNAL OF DAIRY SCIENCE

OCTOBER, 1957

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American Dairy Associations and Affiliations
Heat Effects on Artificially Induced Agglutinins in Milk
Abstracts of Papers Given at Southern Meeting of the American Dairy Science Association

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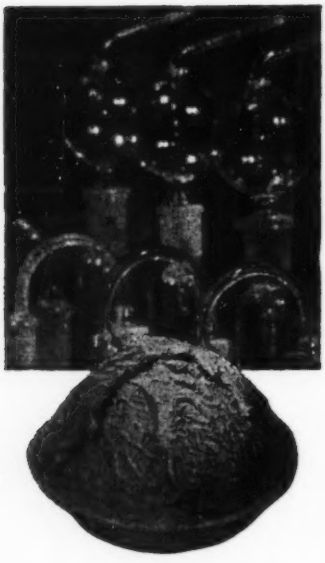

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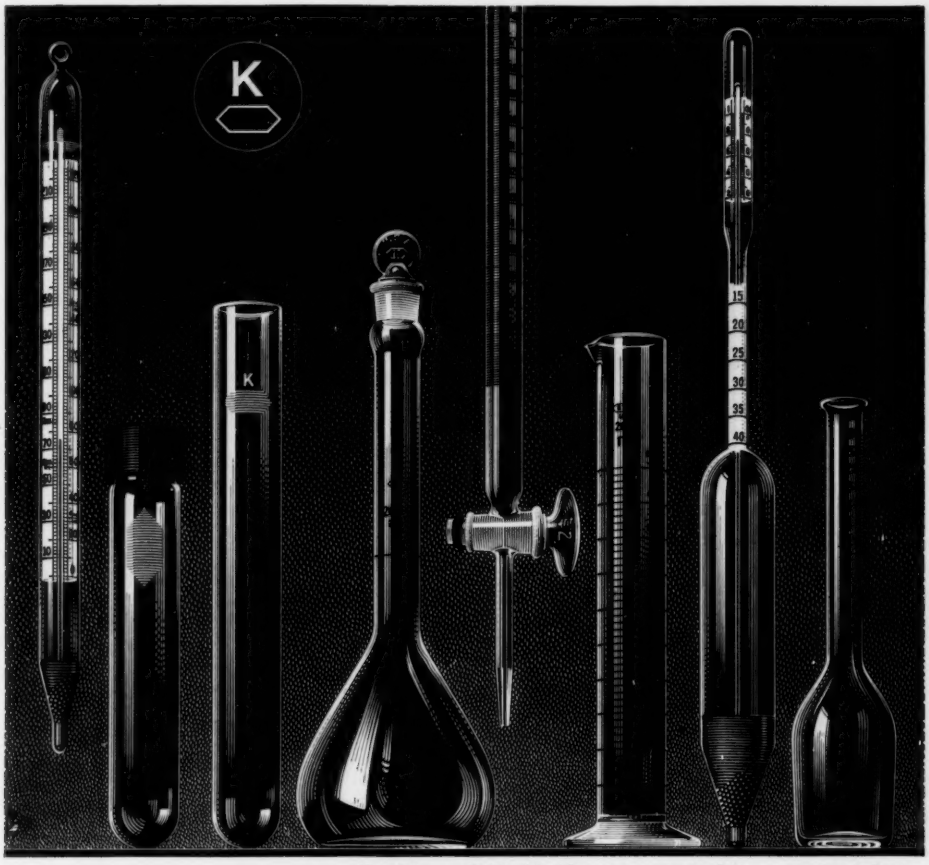

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PEOPLE and EVENTS
in the Dairy Science World

Journal Editorial Changes

At the annual meeting at Oklahoma State Univ., Stillwater, the Editorial Board and Journal Management Committee decided to abolish the editorial foreword on the first page and the terminal summary at the end of research papers. Instead, beginning Jan. 1, 1958, a more complete, factual, and interpretive summary will appear at the beginning of all research papers. This was first suggested by ROBERT JENNESS (*J. Dairy Sci.*, **40**, 16. 1957).

This summary will be convenient to readers of the Journal, reduce costs, expedite the work of abstract journals, and contribute to improved informational services in Dairy Science. It should be a brief and factual summary of the contents of the paper, pointing to new information and its importance. The authors of all research papers are requested to provide a summary according to the following suggestions:

1. Use complete sentences rather than a list of headings. Reference to any author should be in the third person. Standard and not proprietary terms should be used whenever possible. Unnecessary contractions should be avoided. It should be assumed that the reader has some knowledge of the subject but has not read the paper. Therefore, the summary should be intelligible without reference to the paper.
2. The title of the paper is usually read as a part of the summary and for this reason the opening sentence should not repeat the title. If the title is not sufficiently informative, then the opening sentence should indicate the subjects covered. The beginning of the summary should state the objectives.
3. It is sometimes useful to state the treatment of the subject, such as brief, exhaustive, or theoretical.
4. The summary should state new facts, conclusions of an experiment, and, if possible, the essential parts of any new theory, applications, treatments, apparatus, and techniques.
5. The summary should contain names of new compounds, species, etc., quantitative data, and refer to new observations.
6. The summary should indicate the methods used, the basic principles of new methods, range of applications, and degree of accuracy.
7. If it is necessary to refer to summaries of earlier work, the reference should always be given in the same form as in the paper. Otherwise, references should be omitted.
8. The summary should be concise and not exceed 200 words, except in unusual cases. It should be labeled "Summary" and appear as the first paragraph.
9. In addition to the summary, the author may, if he wishes, present concise numbered conclusions at the end of his paper.

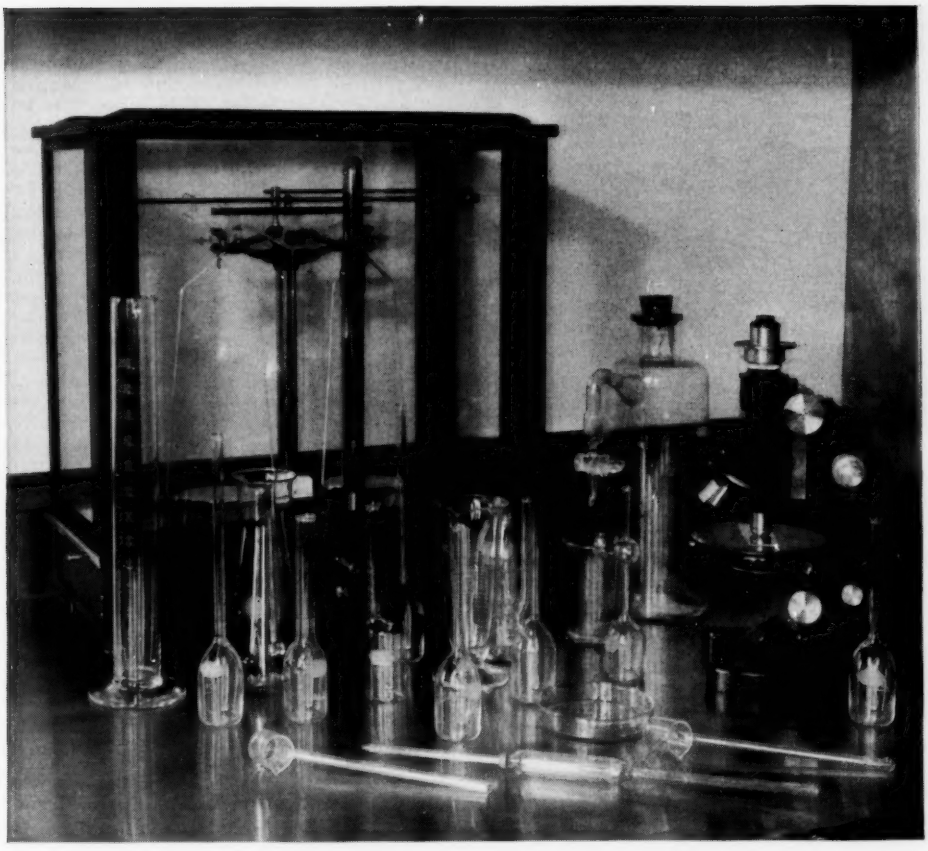
The paper by B. L. LARSON and K. A. KENDALL, *Changes in Specific Blood Protein Levels Associated with Parturition in the Bovine* (*J. Dairy Sci.*, **40**: 659. 1957), was summarized as an example:

SUMMARY

This study elucidates marked decreases in percentages of blood serum protein in the dairy cow which occur at parturition. Periodic blood samples from four cows were withdrawn for 60 wk., which included two parturitions for each animal. Total and nonprotein nitrogen were estimated by semimiero-Kjeldahl analysis. The components of blood serum proteins, i.e., serum albumin, $\alpha 1$, $\alpha 2$, $\alpha 3$, $\beta 1$, $\beta 3$, $\gamma 1$, and $\gamma 2$ globulins, were determined electrophoretically at pH 8.6 (veronal buffer μ 0.1), at 1.2° C. for 5.58 sec. at 7.8 V/cm. Decreases of $\beta 2$, $\gamma 1$, and some α-globulins, which occurred during 4 wk. prepartum, accounted for the 10 to 30% simultaneous decrease in the total serum protein. The levels of serum albumin, $\beta 1$, and $\gamma 2$ globulins did not change appreciably. $\beta 2$ and $\gamma 1$ globulins in the blood serum increased to a maximum about 4 wk. prepartum, then decreased rapidly until parturition. The α-globulins reached a maximum at conception, and thereafter slowly decreased until parturition. These data indicate that $\beta 2$ and $\gamma 1$ globulins of bovine blood decrease markedly toward the end of gestation, when colostrum is being formed in the mammary gland.

In preparing this material, the helpful assistance of W. O. NELSON, Abstract Editor, and ROBERT JENNESS, member of the Editorial Board, is gratefully acknowledged.

E. O. HERREID
Editor-in-Chief



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Gordon to Receive Borden Award in Milk Chemistry

The Borden Award in the Chemistry of Milk, consisting of $1,000 and a gold medal, will go next year to WILLIAM G. GORDON, a supervising chemist at the Eastern Regional Research Laboratory of the USDA, Wyndmoor, Pa.

W. G. Gordon

Choice of Gordon as the 1958 recipient of the award was announced in New York at the 132nd National Meeting of the American Chemical Society. Gordon will thus become one of several chemists working for the Eastern Utilization Research and Development Division of the USDA'S Agricultural Research Service who has received this award. The award has been given annually since 1939.

Gordon is a native of New York City and a graduate of Cornell Univ., Ithaca, N. Y., where he received his M.A. degree in industrial chemistry in 1928. From 1929 to 1935, he was at Yale Univ., New Haven, Conn., where he received his Ph.D. degree, serving successively as teaching assistant, National Research Council fellow in medicine (physiological chemistry), and Coxe fellow in physiological chemistry.

Gordon was an instructor in biochemistry at Stanford Univ., Stanford, Calif., from 1935 to 1939. His research interests during this period centered on the chemistry and metabolism of amino acids.

In 1939 he began his investigations on milk proteins at the Pennsylvania State College, where he served as Smith, Kline, and French fellow until 1941, when he became a research chemist at the recently established Eastern Regional Research Laboratory. Here he continued his research on the modification, isolation, and amino acid composition of milk proteins. Gordon is now in charge of the protein structure unit of the Division's animal proteins section.

Gordon is a member of the American Society of Biological Chemists, American Chemical Society, American Association for the Advancement of Science, New York Academy of Sciences, Sigma Chi, and Phi Lambda Upsilon.

A Message from Your National Membership Committee

GREETINGS TO YOU, FROM YOUR MEMBERSHIP COMMITTEE!

When this message reaches you, school, with all of its many and varied interests and ever-increasing demands on the time of each of you, will have started. Those of you involved in business activities, dairy farming, plant operations, and other agricultural pursuits will be equally busy. However, as in the past, it will be time for all of us to think about and plan for our *National Membership Drive,* which is to be initiated just about the same time as our football season gets under way. We want just as many active participants in this game of membership as possible, but for those who can't be very active in the solicitation campaign, your support from the sidelines will be most appreciated by your Association.

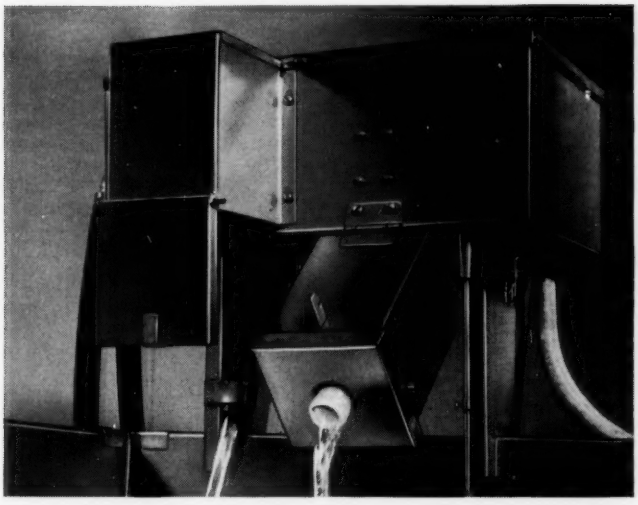
The fine efforts and accomplishments of the membership committee for the past year, under the chairmanship of H. E. CALBERT, is to be commended. The goal of 2,200 members was obtained in spite of the failure of some 200 members to renew their membership. According to the latest records, 358 new members were obtained for 1957 and the membership is currently 2,236. Your committee for the coming year has set a membership goal of *2,400.* This will represent an increase of approximately 10% in membership over the past year. The chairman of each state, region, and division should use the 10% increase as a minimum objective. In some cases, the chairman may feel that it would be desirable to set the goal at a higher level than this.

The student affiliate program has continued to make excellent progress under the leadership of W. L. SLATTER. For example, the student affiliate membership for 1957 is 606 as compared with 531 for 1956. This is an increase of 75, or approximately 14%, in membership. We are confident that the chairman of the student affiliate program for 1958 will furnish the dynamic leadership needed for the continued growth and development of this excellent program. The individual efforts of the membership in this behalf shall be deeply appreciated.

The following dates are pertinent to the membership drive:

Type of member	Initiation of drive	Termination of drive	Clean-up campaign
(1) Student affiliate	Sept. 1, 1957	Nov. 15, 1957	Nov. 15, 1957 to Jan. 15, 1958
(2) Regular	Oct. 1, 1957	Dec. 15, 1957	Jan. 1, 1958 to Feb. 1, 1958

Your committee hastens to point out that, although a concerted effort on membersip will be made by the Association during the above dates, an opportunity to sell the Association to prospective members and to enlist new members at all times should not be overlooked. The matter of active enlistment of new members



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should be a continuous process by each member of the Association.

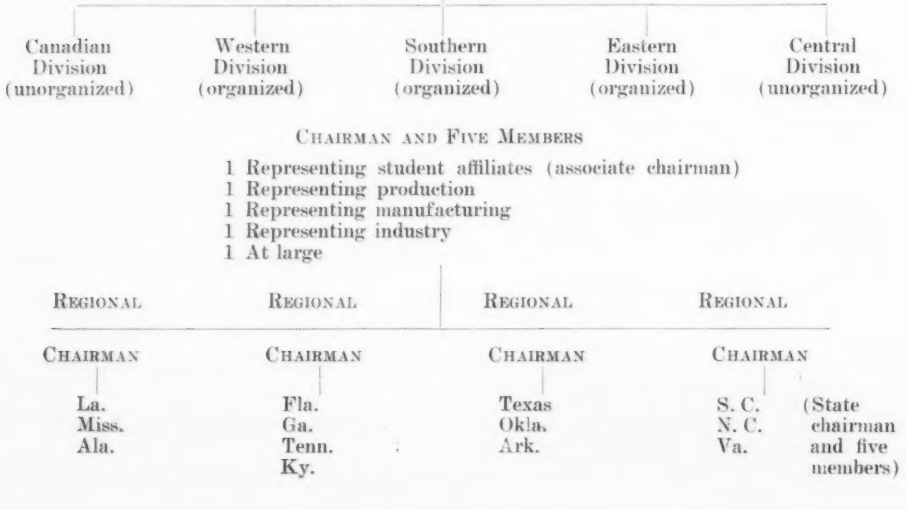
At the annual meeting of the Association in June, the Board of Directors approved a reorganization of the National Membership Committee, in such a way that the various organized divisions of the A.D.S.A. in the different regions would have representation on the national committee and would, thereby, be more effective in promoting membersip. It is felt that if the Southern, Western, and Eastern Divisions emphasize membership at their annual meetings and, that if the efforts of the Divisional Membership Committee are coordinated with those of the national committee, a tremendous increase in effective members of the A.D.S.A. may be realized within the next few years. In view of the shortage of time, the Southern Division is the only one that is being organized for the ensuing campaign according to the new plan presented herein. If this plan proves effective, it is hoped that it can be initiated in the other divisions for the membership campaign of 1959.

It is noted from the diagram that the associate chairman for the national and divisional committees would be in charge of the student affiliate membership program. The purpose of this arrangement is to provide more coordination between the membership program for regular members and student affiliates. The National and Divisional Committees have the responsibility of organizing, planning, and conducting the membership campaign each year. This could be done easily at the time of the annual meetings of each Division and of the A.D.S.A. Members of the Divisional Committee (other than the associate chairman) would be designated as Regional Chairmen and would be charged with the responsibility of carrying out the membership campaign in accordance with the plans adopted by the over-all committee.

The membership committee needs your assistance. Just think of what it would mean to the strength and progress of our Association if each member obtained one new member for 1958. I am sure that each member knows personally at least one person who should belong to the A.D.S.A. Such personal acquaintance is the most effective means by which such people can be brought into the fold. Are you willing to do your part? Wouldn't it be a good feeling to be able to look back, when the campaign is over, and say, "Yes, I helped with the membership campaign by personally obtaining one new member." Yes, it is a worthwhile effort and really it won't take much time! Give it a try, won't you, and I'm sure we all will be happy with the results. Please remember that your committee will be working and will be happy to give you any assistance needed in your individual efforts.

There would be a National, Divisional, and Regional chairman, and one for each state, also an associate chairman at the National and Divisional level. The Associate Chairman would be the person selected as the student affiliate representative on the committee. Also, the Associate Chairman at the National level should be associated with the student affiliate program. Members of the Divisional Committee (other than the Associate Chairman) would be designated as regional chairmen and would be responsible for

ORGANIZATION FOR MEMBERSHIP DRIVE FOR A.D.S.A., 1958



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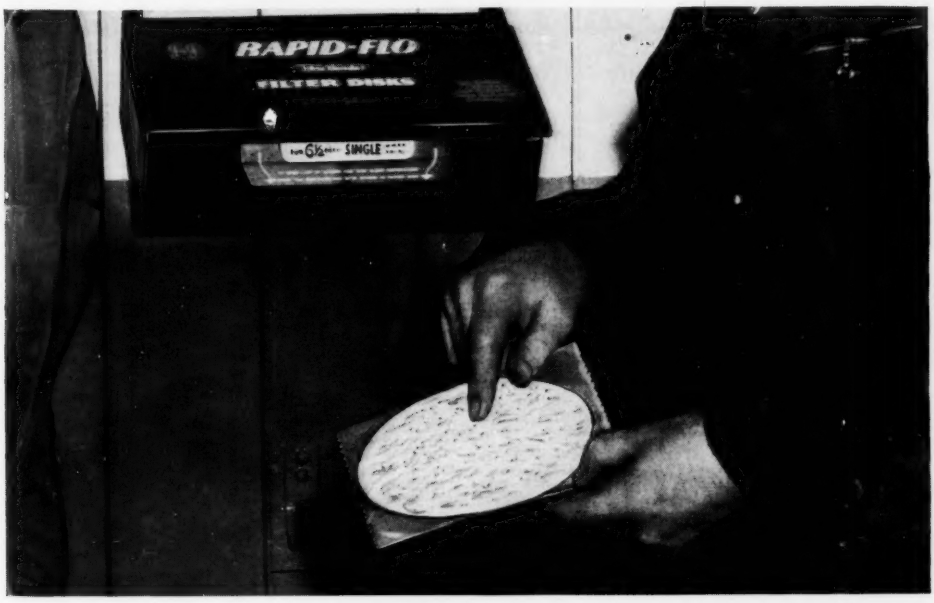

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carrying out the membership campaign in accordance with the plans adopted by the over-all committee.

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DR. J. E. MOXLEY, McDonald College of McGill Univ., Quebec, Canada

University of Georgia News

FRANK W. FITCH, Extension dairyman since 1920, retired July 1, 1957.

Mr. Fitch was the second secretary of the Georgia Jersey Cattle Club formed in 1918, the oldest continuously active breed association in the state. He served in that position for over 20 years. He was identified with every phase of dairying, leading in the development of interest in dairying among farm boys and girls and training several leading 4-H Club teams for national contests.

Mr. Fitch assisted in selecting the foundation herd of Jerseys for the Georgia State Prison at Reidsville, said to be the only herd ever assembled in which all of the cows classified Very Good or Excellent. He led in establishing artificial insemination of dairy cattle in the state and ably supervised its progress. He served as secretary and directed the publicity of the State Brucellosis Committee.

In 1948, Mr. Fitch was elected an honorary life member of the Georgia Student Branch of the American Dairy Science Association. In the same year he was selected by the Georgia Dairy Association as Man of the Year in Dairying.

STEVEN J. SPECK, for seven years assistant dairyman and superintendent of the University Creamery, resigned his position, effective August 15, 1957, to accept the directorship of quality control for Irvindale Farms Dairy, one of the leading milk distributors in Atlanta. Mr. Speck was an adviser of the Dairy Science Club and for several years supervised the publication of the Clubs' annual by the student members.

OLIN T. FOSGATE joined the staff of the Dairy Dept. on September 1, 1957, as assistant professor of dairying. Half of his time will be devoted to teaching and half to research in the production field. He succeeds GERALD H. STOTT, who recently became a member of the staff of the Dairy Dept. of the Univ. of Arizona, Tucson. Dr. Fosgate was formerly associated with the William H. Miner Agricultural Research Institute in New York. He obtained his B.S. and Ph.D. degrees at the Univ. of Wisconsin, Madison, specializing in the physiology and breeding of dairy cattle. At that institution he was also an assistant in teaching and in research at the Emmons Blaine, Jr. Experimental Farm.

ROBERT E. ROBERTS, a dairy alumnus of the University, became assistant dairyman and superintendent of the University Creamery on September 1, 1957.

Construction is under way on the University's multimillion-dollar Science Center. Funds for its completion will become available as construction progresses.

The Dairy Science Club of the Univ. of Georgia, Athens, was awarded one of the three Certificates of Merit for its club activities, at the annual meeting of the American Dairy Science Association. The officers during the year were T. W. MCKINLEY and JOHN JENKINS, presidents; RICHARD BISHOP and CHARLES WALLACE, vice-presidents; IRA KATZ and JAMES CLARK, secretaries; RICHARD BISHOP, treasurer; and JOHN JENKINS and LARRY YOUNG, publicity directors. Each served for one-half of the year. H. L. DALTON is faculty adviser.

Ohio State News

Winners in the various classes of the Dairy Products Division at the 1957 Ohio State Fair were: *Butter Workmanship*, VIRGIL HOWDYSHELL, Pickerington Creamery, Pickerington; *Creamed Cottage Cheese*, WARREN POLK, Borden's, Springfield; *Swiss Cheese in Blocks*, PAUL BURKEY, Burkey Dairy, Sugarcreek; *Swiss Cheese in Wheels*, ERNEST SCHMID, Sharp Run Dairy, Millersburg; *Milk Judging*, ROBERT OBERLIN, Massilon; *Ice Cream Judging*, BRUCE SHIVELY, Columbus; *Butter Judging*, CHARLES NEIKIRK, Bucyrus, *Cheese Judging*, ARNOLD LUDWIG, Toledo.

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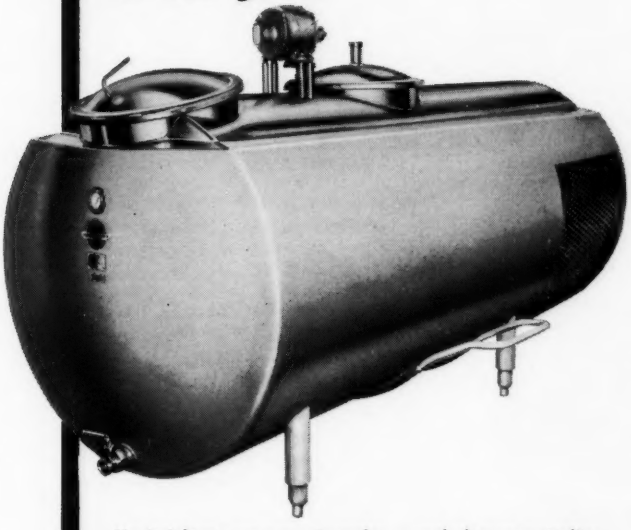

Use it right now as a pour-in cooler . . . and when your pipeline comes along you're all set with the most advanced Vacuum Bulk Cooler on the market. This new 375-gallon, self-contained, factory-sealed Vacuum Bulk Cooler is exclusive with De Laval . . . has the same built-in quality and dependability that make every De Laval Bulk Cooler outstanding.

Perfect companion for a pipeline milker, the new De Laval Vacuum improves milking . . . eliminates the need for milk handling equipment—pump or automatic discharger . . . makes less *total* cost for Combine Milker and tank.

Get all the money-making facts on De Laval's new Vacuum Bulk Cooler . . . and on De Laval's other bulk cooler models. There's a size to fit your operation perfectly—every-day or every-other-day pickup—capacities from 180 gallons to 1000 gallons. See your De Laval Dealer . . . today!

NEW DE LAVAL Self-Contained Bulk Cooler . . . automatic, trouble-free . . . capacities from 180 to 375 gallons.

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The official judges were R. E. HARDELL, Monroe, Wis., for Swiss cheese, and PROF. J. M. JENSEN, Michigan State Univ., East Lansing, for all other classes. The various contests attracted over 100 entries.

The ninth annual Dairy Technology Homecoming Brunch was held Nov. 2.

D. H. JACOBSEN, Research Director, The American Dairy Association, spoke on "The Role of Research in the Dairy Industry" at the October meetings of the Cincinnati, Central Ohio, Northeastern Ohio, and Maumee Valley Dairy Technology Societies.

A series of five Fieldmen's meetings has been planned for November in Columbus, Middletown, Findlay, Akron, and Cambridge. The committee in charge of the meetings includes DICK BUEHLER, M & R Dietetics, DICK STAFFORD, Diamond Milk Products, and RALPH PALMER, Borden's, all of Columbus. The Committee is working in close cooperation with the Ohio State Agricultural Extension Service.

The following changes of position have been reported by Ohio State Univ. graduates in Dairy Technology: CARMEN J. FOX '50, manager of Youngstown Sanitary Milk Co., Youngstown; DALE GILLESPIE '39, manager of the eastern zone of Creamery Package Manufacturing Co.; LOU KOMMINSK '39, Vice-President of Beatrice Foods Co.; CHARLES FISHER '45, production chief, Borden's Ice Cream Plant, Columbus; RAY ALBERTS '39, sales manager, Potlach-Forest, Inc., Chicago; TOM WILSON '55, administrative assistant, Ohio Dairy Products Association, Columbus; DON VELL, M.Sci. '57, quality control, Kroeger, Manion, Ind.; O. B. CURLEY, M.Sci. '55, sales representative, Klenzade, San Francisco.

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News from Wisconsin

L. H. SCHULTZ, formerly Professor of Dairy Husbandry at Cornell University, Ithaca, N. Y., has become a member of the Dairy Husbandry staff at the Univ. of Wisconsin, Madison.

He replaces VEARL R. SMITH, who is now Chairman of the Dept. of Dairy Science at the Univ. of Tucson, Arizona.

Dr. Schultz has conducted research primarily in the field of the physiology of milk secretion, which he will continue to pursue at Wisconsin, in addition to teaching Dairy Husbandry courses.

He received his B.S. from Wisconsin in 1941 and his Ph.D. in 1949; since then he has been engaged in research and teaching at Cornell Univ., Ithaca, N. Y. He is a member of the American Dairy Science Association, American Society of Animal Production, and Alpha Zeta and Phi Kappa Phi honorary fraternities.

Tennessee News

Recent additions to the Dairy Dept. staff at the Univ. of Tennessee, Knoxville, are B. J. DEMOTT, Associate Professor, specializing in dairy technology; R. G. CRAGLE, Associate Dairy Husbandman, who will be working with the UT-AEC Agricultural Research Program, and J. J. SULLIVAN, a part-time instructor in Dairying.

Refugee Seeks Position in the U. S.

BOLDIZSAR NEMETH, a Hungarian refugee now living in England, is interested in a position in this country. He has had considerable experience in the field of artificial insemination and is a specialist in livestock breeding. If he obtains a position, he could be included in the first preference quota to enter the United States as an agricultural specialist. Dr. Nemeth speaks German, can express himself fairly well in English and French, and is studying English more intensively. He has excellent references. Dr. Nemeth states, "I would do my utmost to work well if you could only help me start a new life in your great country."

Anyone interested, can obtain further information from the Editor, *Journal of Dairy Science.*

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dynamic DYNA-FOS dicalcium phosphate supplies

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Twenty years ago, a pig did well to weigh 140 lbs. at 5 months. Today he tops 200 lbs. in the same time — does it on less feed per lb. of gain.

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At Purdue, swine nutritionists boosted pig gains as much as ½ lb. per head daily . . . just by replacing soft phosphate with dicalcium phosphate. And the pigs made each lb. of gain from nearly ½ lb. less feed.

Other college tests show similar results. They're proof that chemically produced dicalcium phosphates such as International's Dynamic DYNA-FOS meet the need for a harder-working phosphorus in today's livestock and poultry rations.

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Learn about feed phosphorus sources in this FREE booklet which gives many useful facts about phosphorus. Write Technical Service Department . . .

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Kansas State News

The Fifth Regional Intercollegiate Dairy Cattle Judging Contest was held at Hutchinson, Kansas, in September during the Kansas State Fair, with eight teams competing.

The Kansas State Board of Agriculture Trophy for the winning team was won by the team from Iowa State College, Ames. The members of this team were RONALD JOHNSON, JOHN KRAMER, and WILLIAM WUNDER, and the coach was C. F. FOREMAN. The seven other competing teams ranked as follows: Kansas State College, Manhattan; South Dakota A & M, College Station; Univ. of Nebraska, Lincoln; Univ. of Missouri, Columbia; Univ. of Arkansas, Fayetteville; Oklahoma State Univ., Stillwater, and Colorado A & M, Ft. Collins.

The high individual in the contest was CHARLES MICHAELS of Kansas. Runner-up was RONALD JOHNSON, Iowa, followed by FRED BEMENT, South Dakota.

The teams were banquet guests of the Kansas Cooperative Dairy Products Association and the Kansas State Dairy Association. Prizes were awarded in the breed divisions by the American Guernsey Cattle Club, American Jersey Cattle Club, Brown Swiss Cattle Breeder's Association, Ayrshire Breeders' Association of America, Holstein-Friesian Association of America, and Milking Shorthorn Society of America. Breed organization representatives selected and placed cattle of their breeds and scored contestants. KEITH HUSTON was superintendent of the contest.

A million-dollar fire in August destroyed the east wing of Waters Hall, which houses the School of Agriculture. The central and west portions, which include the Dairy Department facilities, were not damaged.

ROSS MICKELSEN, who received his M.S. degree in August from Utah State College, Logan, has joined the Dairy Husbandry staff at Kansas State and will be engaged in teaching and research in Dairy Manufacturing.

A Kansas State co-ed, 19-year-old BARBARA JEAN DAVID, Winfield, was crowned Kansas Dairy Princess at the State Fair in September. She will represent Kansas at the 1957 American Dairy Princess contest in Chicago. Among the awards she received were a $300.00 scholarship from the Kansas Ice Cream and Milk Institute, a wardrobe from the Kansas Dairy Association, and luggage from the Kansas Butter and Cheese Institute.

Louisiana State News

B. L. KILPATRICK, formerly assistant county agent in Franklin Parish, has been named assistant dairy specialist, Louisiana Agricultural Extension Service, to fill the vacancy made by the retirement of R. C. CALLOWAY.

Mr. Kilpatrick was born in Louisiana and received his B.S. and M.S. degrees at Louisiana State Univ., Baton Rouge, where he majored in dairying. He spent 18 months overseas, working in Germany with the veterinary branch of the Army. He is a former 4-H Club member and at that time served as president of the Ouachita Parish 4-H Executive Council.

His work will be concerned with assisting the breeding and testing organizations and holding field days, sales, and group classifications. He will serve as judge at district and parish shows and will act as superintendent of dairy cattle at the state shows.

A new record will be set this year by Louisiana for the number of dairy cows bred in the artificial insemination program. A. D. ANDERSON, associate dairy specialist, said the number would increase to 44,900. Last year 40,913 cows were bred.

E. W. NEASHAM, Extension dairyman, at a state-wide conference of breeding technicians, said that 47 breeding units are now operating in 60 of the 64 parishes in the state. Headquarters are at the L.S.U. Dairy Improvement Center. It is now possible for the dairymen having only a small business to have his cows bred to some of the best bulls in the United States.

DR. J. B. FRYE, JR., head of the Dairy Dept. of Louisiana State Univ., Baton Rouge, who since 1952 has been advising with the Venezuelan government regarding the development of the dairy industry in that country, returned recently from his last visit there. His work has been especially concerned with the development of programs favorable to the growth of dairying and the production of better quality milk.

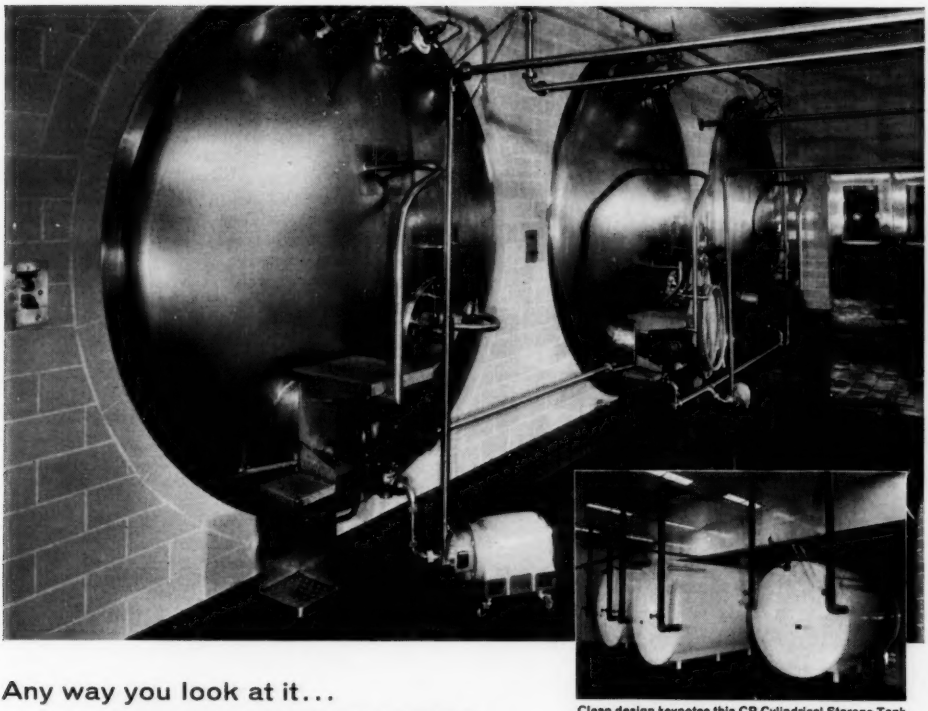
On his first trip to Venezuela Dr. Frye made a special study of the dairy situation in the Caracas milk shed. Since then he has returned several times for further consultation with government officials and in 1957 he spent a month making recommendations with special emphasis on ways of increasing production, improving quality of milk, and increasing the efficiency of production.

As a result of these studies and experiences he has written several bulletins on dairying in Venezuela. These bulletins, published in Spanish, have been given wide distribution throughout the country.

V. P. I. News

P. M. REAVES, Professor of Dairy Science, Virginia Polytechnic Institute, Blacksburg, was one of three V.P.I. faculty members to receive the first W. E. WINE Awards for faculty achievement. Along with this award, Prof. Reaves received a check for $500 from the V.P.I. Alumni Association.

The cash awards were established last year to recognize contributions to the teaching profession made by faculty members in V.P.I.'s



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Clean design keynotes this CP Cylindrical Storage Tank installation at Fred Meyers, Inc., Portland, Ore. Shown are one 2500-gallon and two 5000-gallon sweet water-cooled tanks, for incoming raw milk. Wheeled pump in foreground (large picture) is a CP Centrifugal Stainless Steel Sanitary Pump.

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The Inside—here you'll see a heavy stainless steel inner lining CP precision welded, ground and polished to provide a clean unobstructed surface for your product.

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CP Storage Tanks are available in cylindrical or rectangular models, cold-hold or refrigerated models (direct expansion or sweet-water) in 1000 to 10,000 gallon sizes.

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three schools: Agriculture, Applied Science, and Engineering. The Agricultural award went to Professor Reaves. Nominations for this award were made by the students and faculty, and final selection of the recipient was made by the executive committee of the Agricultural Faculty.

Professor Reaves received his undergraduate and graduate degrees from the Univ. of Tennessee, Knoxville, and joined the staff of the Dept. of Dairy Science at V.P.I. in 1928. He is co-author of the college textbook *Dairy Cattle Feeding and Management;* has served as faculty adviser of the Virginia Tech Chapter of the A.D.S.A. for a number of years, and also as adviser to other student organizations, and he teaches classes in feeding, breeding, and management of dairy cattle, and public relations in dairying.

Cheese Conference at Beltsville, Md., Oct. 30-31

A Research Conference on cheese was held Oct. 30-31 at Beltsville, Md.

The following topics were discussed: Functions of Food and Drug Administration, by W. Horwitz; Starter Developments, by M. W. Hales and F. J. Babel; Starter Failures, by R. P. Tittsler and R. L. Winslow; Treating Milk for Cheese with Hydrogen Peroxide, by Z. D. Roundy; New Methods for Making Italian Cheese, by F. V. Kosikowsky; Manufacturing High Quality Cottage Cheese, by M. E. Powell; New Method for Making Cheddar Cheese, by H. E. Walter; Utilizing Whey in Food Products, by C. A. Alesch, and Chemistry of Cheese Flavor, by W. J. Harper.

A demonstration of making Cheddar Cheese by the Short-Time Method was conducted by H. E. Walter, A. M. Sadler, and C. D. Mitchell.

This conference was organized by Dr. P. A. Wells and his associates at the Eastern Utilization and Development Division, USDA, in cooperation with directors of Eastern Agricultural Experiment Stations.

Soft-Served Dairy Products Short Course at Illinois

The Dept. of Food Technology at the Univ. of Illinois, Urbana, held a short course on Soft-Served Dairy Products, Nov. 6-7, in the Illini Union bldg.

Topics discussed were: Laws Concerning Store Operation; How to Be Successful in Business; Do You Know Your Costs; New Outlets for the Soft-Served Store, and Insect Control.

Faculty members who participated in this conference were: L. B. Howard, Dean of Agriculture; R. T. Milner, Head of the Food Technology Dept.; W. N. Bruce, F. H. Beach, R. M. Nolen, J. Tobias, S. L. Tuckey, P. H. Tracy, and L. D. Witter.

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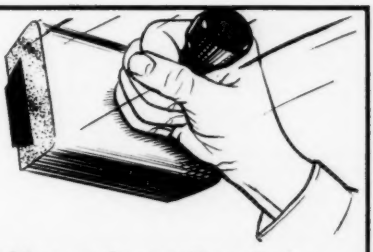

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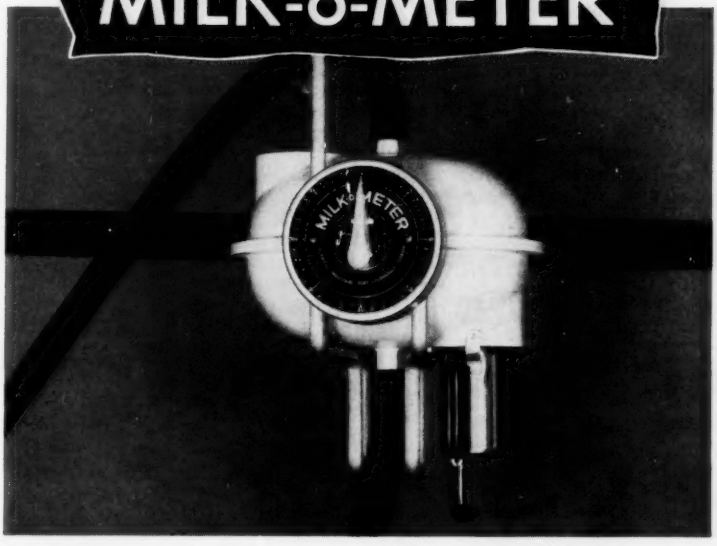

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News from Borden's

Appointment of M. FAIRMAN as an assistant vice-president of the Borden Company, in charge of advertising and public relations, has been announced. Mr. Fairman has been director of public relations since 1943. W. B. CAMPBELL was promoted from manager of the general advertising department to director of advertising. Both appointments became effective Oct. 1.

The promotions followed the announcement that STUART PEABODY will retire as an assistant vice-president on Dec. 31, after 33 years' service as a Borden advertising executive. Mr. Peabody became an assistant vice-president in 1946. He has held executive responsibility for advertising and public relations and has also been a director of advertising, a post which he first assumed in 1930. Mr. Peabody was chairman of the Advertising Council for two years, and is a former president of the Association of National Advertisers and a former chairman of the Advertising Research Foundation. He was awarded the Gold Medal Career Award for 1954, an annual advertising award sponsored by *Printer's Ink*.

Mr. Fairman, a former Chicago newspaperman, joined the Company in 1937 and organized its regional public relations office in Columbus, Ohio. He remained there as regional public relations director for six years. In assuming his post as an assistant vice-president, Mr. Fairman will continue as director of public relations. He is a former president of the Public Relations Society of America and a former editor of the *Public Relations Journal*.

Mr. Campbell joined Borden's in 1951 and that same year became assistant radio-television advertising manager. He was named national advertising manager for radio and television in 1954, and early this year became manager of the general advertising department.

Kentucky's Fifth Annual Short Course

Dairy leaders from all parts of the United States will meet in Kentucky to discuss dairy problems at the Fifth Annual Manufacturing Short Course at the Univ. of Kentucky, Lexington, Dec. 3-5. The short course is sponsored jointly by the Dairy Section of the Univ. of Kentucky and the Dairy Products Association of Kentucky.

Ice cream, market milk, and cheese each will be given a day at the course, with the problems unique to each being discussed at that time. The ice cream program will be concerned with how to produce good ice cream.

Present problems and the future of the market milk industry will be discussed the second day, and Dec. 5 will be devoted to problems peculiar to the cheese industry. Outstanding

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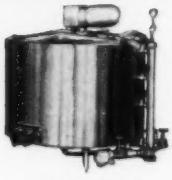

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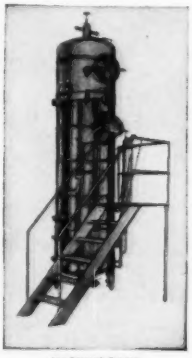

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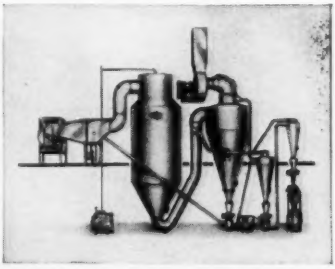

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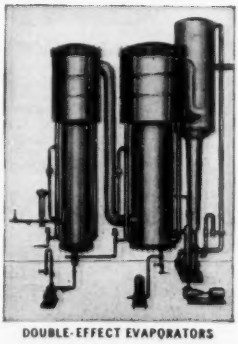

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Engineered Heating Equipment
for flavor control, flexibility, operating economy

experts in all phases of the dairy field have been engaged for the course.

Clinics in ice cream, cottage cheese, buttermilk, and Cheddar cheese will continue to be a feature of the short course. A new feature this year will be a milk-scoring contest, open to those attending the short course.

The annual short course banquet will be held Dec. 4, with J. G. HAYES, Michigan State Univ., East Lansing, as the banquet entertainer. Fee for the three-day course will be $10, which includes the banquet ticket. Further information concerning the short course may be obtained by contacting A. W. RUDNICK, JR., chairman of the short course, Dairy Section, Univ. of Kentucky, Lexington.

Nations Seeking Greater Import Controls

Five additional nations currently are seeking to tighten up their imports of foreign commodities, for the benefit of their local industries, according to a recent U. S. State Department announcement. The countries involved are Austria, Canada, Ceylon, Greece, and the Union of South Africa. They seek to invoke tighter controls by modifying concessions which they made under the General Agreement on Tariffs and Trade.

In the meantime, many other nations are using import controls. In countries without freely convertible currency, the method is by trade and exchange controls. In many others—including Canada, Mexico, and Venezuela—the method has been that of quantitative import controls similar to dairy import controls in the U.S. Among Latin-American countries, there are many instances of more or less complex multiple-exchange-rate systems. Brazil, for example, has had a system of five import action categories, requiring importers of some farm products (including butter) to pay premiums so high as to preclude imports. Examples, it is said, could be multiplied the world over.

The entire foreign trade policy of the U.S.—including the import control program—is scheduled for congressional review next year, before the expiration of the current Reciprocal Trade Agreements Act.

Hungry Days Predicted by Professor

PROF. GEORGE BORGSTROM of Michigan State Univ., East Lansing, recently gave the staff of Agriculture Research Service's Eastern Research Laboratory a gloomy picture of seriously lowered nutritional standards some year ahead.

He said, "Although only a fraction of the energy produced by plants in photosynthesis is now converted into human energy through digestion, it will be difficult for the world's population to derive much more nutrition from Nature." If the United States continues its present population expansion, he doubts that it will be able to maintain its present standards of feeding for long.

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Polish Agriculturalists Visit U. S.

A delegation of Polish agricultural specialists visited the United States in September for a month-long survey of American farm achievements. The five-member delegation was the first of its kind to come here in more than a decade.

Chairman of the group is Professor JAN KIELANOWSKI, director of the Institute of Animal Physiology of the Polish Academy of Sciences. The other members of the delegation are JAN BOJANCZYK, director of the Central Board for the Mechanization of Agriculture in the Polish Ministry of Agriculture; BOGUMIL SZERSZEN, Chief of the Division of Fertilizer Husbandry of the Ministry of Agriculture; TADEUSZ RUEBENBAUER, a corn expert on the faculties of the Higher Schools of Agriculture in Wroclaw and Krakow and a member of the Council of the Polish Ministry of Higher Education, and EUGENIUSZ PIJANOWSKI, Director of the Chair for Food Industries at the Central Agricultural College in Warsaw, which conducts an Institute for Milk Technology and an Institute for Fruit and Vegetable Techniques.

During their visit in the United States, the Polish agricultural scientists met with officials of the U.S. Department of Agriculture, and with other farm experts, in Iowa, Wisconsin, and Illinois.

Completed Theses

Ph.D. Degree

GABRIEL BIALY—Studies of physiology of bovine spermatozoa. Univ. of Wisconsin, Madison.

M.S. Degree

VERNON COLENBRANDER—Studies of calcium and phosphorus metabolism using chelating agents. Univ. of Wisconsin, Madison.

JAMES D. HARRIS, JR.—Observations of Jersey and Holstein calves with special attention to rumination and rumen development. Univ. of Tennessee, Knoxville.

B. R. MAUK—A study of some factors influencing the rennet curd tension of milk and casein sols. Univ. of Tennessee, Knoxville.

WILLIAM M. MILLER—Feeding thyroxine and thyroactive protein to milking cows. Univ. of Tennessee, Knoxville.

B. PARKINS—A study of calf-raising methods using reconstituted skimmilk. Univ. of Tennessee, Knoxville.

JAMES C. PROW, JR.—The growth response of coliform bacteria in milk to an increase of 5° C. Univ. of Tennessee, Knoxville.

JAMES A. ROBERTSON, JR.—Studies of milk lipase in bovine mammary tissue and separator slime. Univ. of Tennessee, Knoxville.

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Young Joins Florida Staff

HOWARD B. YOUNG has accepted a position with the Extension Division of the Univ. of Florida, Gainesville, in the field of Dairy Manufacturing.

H. B. Young

He graduated from the Univ. of Georgia, Athens, in 1950 with a B.S. degree, majoring in dairy manufacturing, and with a M.S.A. degree in the same field, from the Univ. of Florida in 1957.

He is a member of the Alpha Gamma Rho social fraternity and Phi Sigma honorary biological fraternity. He served for three years in the U. S. Navy and has worked in commercial dairies, supervising operations for about eight years.

Mr. Young will work with the dairy manufacturing interests of the state, dealing with problems in the processing and manufacture of dairy products.

Eastern Division of A.D.S.A. Meets

The 31st annual meeting of the Eastern Division, American Dairy Science Association, was held at the main office of the Eastern States Farmers' Exchange, Inc., West Springfield, Mass., Sept. 16. Abstracts of the papers will appear in the Journal.

The intercollegiate judging contest, sponsored by the Eastern States Exposition, drew six teams in products judging and 14 teams in cattle judging. The team from the Univ. of Connecticut, Storrs, won first place in the judging of all products, and the team from Cornell Univ., Ithaca, N. Y., first place in the judging of all breeds of cattle. Awards to the judging teams were presented at an awards banquet sponsored by the Eastern Division, held September 17 in Springfield, Mass.

The new officers of the Eastern Division, who took over at the banquet, are R. E. WARD, Eastern States Farmers' Exchange, West Springfield, Mass., *chairman;* A. C. SMITH, Dept. of Animal Industry, Univ. of Connecticut, *vice-chairman;* B. R. POULTON, Dept. of Animal Industries, Univ. of Maine, Orono, *secretary-treasurer.*

Nonfat Dry Milk to Go to Puerto Rico

Purchase of 2.7 million lb. of nonfat dry milk for Puerto Rico has been announced by the USDA. The product is for donation to school-lunch and welfare use. Half was delivered by November 1 and the remainder will be sent to

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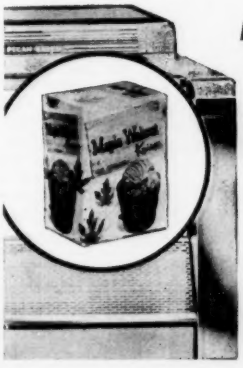

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Puerto Rico by November 29. The prices were 20.43 and 20.44 cents per pound.

Valuable Cattle to Rutgers University

JAMES P. BENDER, treasurer of the Bethlehem Steel Co., has given to Rutgers Univ., New Brunswick, N. J., 90 head of dairy cattle worth an estimated $30,000.

The last of the 60 Holsteins and 30 Guernseys were transferred this week from Mr. Bender's farm near Coopersburg, Pa., to the University's dairy research farm, Beemerville.

Announcing receipt of the gift, JOHN W. BARTLETT, chairman of the Rutgers Dairy Dept., commented that the Holsteins will be particularly valuable for research, because they carry the same blood lines as cattle at the dairy research farm.

He said it was Mr. Bender's wish that his gift would enhance the value of the long-time breeding studies of the Dairy Dept. Mr. Bender has been breeding his Holstein cows artificially to bulls at Beemerville for 15 years.

The University's benefactor is a brother of CARL B. BENDER, Cranbury, many years a member of the Rutgers faculty. Upon retirement, he was given the title of Honorary Professor of Grassland Farming.

APHA to Meet in Cleveland, Nov. 11

Reports on a wide variety of research projects, aimed at protecting and advancing human health, will highlight the 85th annual meeting of the American Public Health Association and meetings of 40 related organizations, in the Cleveland Public Auditorium, beginning Nov. 11.

More than 5,000 public health workers are expected to attend, according to REGINALD M. ATWATER, executive secretary of the Association.

Of about 400 scientific papers to be presented in 75 sessions, more than half will be reports on research accomplishments or opportunities.

There is general realization of the fact that yesterday's methods will not be practical in coping with tomorrow's health problems. This year's APHA annual meeting will give research workers an opportunity to share their findings with other health specialists whose job it is to translate research into action programs aimed at promotion of personal and community health, the maintenance of a healthful environment, and an aggressive attack on disease and disability.

The four general sessions scheduled are:

Nov. 11—A symposium on public policy aspects of public health planning; Nov. 12—Evening session featuring addresses by the Association's president, JOHN W. KNUTSON, assistant surgeon general and chief dental officer of the U. S. Public Health Service, and LOWELL

J. REED, president emeritus of Johns Hopkins Univ. The Sedgwick Memorial Medal will be presented at this session; Nov. 13—Lasker Awards of the American Public Health Association for 1957 will be presented Thursday evening, Nov. 14—A closing symposium Friday afternoon will focus on public health priorities for 1958.

The American Public Health Association is the largest professional organization of public health workers in the western hemisphere. Its more than 13,000 members include physicians, dentists, nurses, veterinarians, engineers, sanitarians, statisticians, nutritionists, entomologists, biologists, health educators, and other specialists in public and voluntary agencies and in private practice.

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COBALT AND VITAMIN $_{12}$ IN RUMINANT NUTRITION: A REVIEW

S. E. SMITH AND J. K. LOOSLI
Dept. of Animal Husbandry, Cornell University, Ithaca, N. Y.

This review will not delve into details of either cobalt or vitamin B_{12} metabolism, since these separate subjects have been extensively reviewed elsewhere. Cobalt in the nutrition of animals has been reviewed by Russell *(61)*, Maynard and Smith *(51)*, Beeson *(10)*, Marston *(48)*, and Underwood *(72)*, as well as others. Vitamin B_{12} in its many aspects has been reviewed by Zucker and Zucker *(77)*, Smith *(63)*, Ungley *(73)*, Marston *(48)*, Sebrell and Harris *(62)*, Ford and Hutner *(26)*, and Williams *(76)*.

Cobalt. A devastating syndrome in ruminants, now recognized in many parts of the world, was demonstrated in 1934 to be the result of cobalt deficiency, first in pastures of certain areas, then in harvested forages, and later in selected mixed rations. The nonspecific symptoms, demonstrated by many different groups of workers, included inappetence, followed by a loss in production and an anemia. The anemia has been described by Smith *et al. (65)* to be normocytic and normochromic, and by Marston *(48)* to be macrocytic. A recheck of the type of anemia, by Smith *(64)*, confirmed the earlier observation of a simple anemia, so it appears that there must be some fundamental difference between the cobalt deficiency observed by the New York workers and the Australian group. Postmortem findings in cobalt-deficient sheep have not been helpful in differential diagnosis, since the pathological alterations are typically those of general inanition, sometimes accompanied by a fatty degeneration of the liver and hemosiderosis of the spleen. Depraved appetite has sometimes been cited as a symptom of cobalt deficiency. In our observations in pen-raised sheep over a period of approximately eight years, involving over 400 animals, depraved appetite has never been a prominent sign of this deficiency. Apparently, this is in agreement with Marston's extensive observations, for he has not cited an abnormal appetite as being characteristic of cobalt deficiency under Australian conditions.

Since the anemia of cobalt deficiency characteristically follows the anorexia, and is the last symptom to return to normal, or approximately so, after cobalt therapy, it has been frequently stated that the anemia is probably not the primary sequela but is, rather, secondary to appetite failure. It will be recognized that this statement denies any direct action of cobalt on hemoglobin and/or erythrocyte formation. Since there is no conclusive evidence one way or the other on this point, it might more properly be left open, and thus serve as a challenge to others to delve into the problem in more detail than has been done in the past. Beyond this, the anemia has other interesting facets. Observations are in general agreement that the anemia of cobalt deficiency responds slowly and variably following either cobalt or vitamin B_{12} administration, so much so that as a criterion of treatment-response we consider it secondary to appetite-response. More often

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than not, hemoglobin never does quite reach normal levels following therapy, which suggests that some now-unrecognized factor is lacking.

Several lines of evidence early indicated that cobalt functioned in some manner associated with the ruminant stomach. There were early reports that nonruminants, horses and rabbits, behaved normally on pastures that were cobalt-deficient for sheep and cattle. Laboratory animals, rats [Houk *et al. (34)*], and rabbits and guinea pigs [Thompson and Ellis *(70)*], failed to develop a cobalt deficiency, even though fed diets containing much less cobalt than cobalt-deficient pastures. Further evidence was furnished by varying the route of administration of cobalt salts. As early as 1944, McCance and Widdowson *(47)* mentioned a personal communication to the effect that cobalt given parenterally to cobalt-deficient sheep was not therapeutically effective. Marston and Lee *(49)* published data demonstrating that 1 mg. of cobalt fed per day to deficient lambs was therapeutic; whereas, the same amount given parenterally was not. This was confirmed by Smith *et al. (65)*. On the other hand, Keener and Percival *(37)* and Ray *et al. (57)* reported slow responses in deficient lambs given cobalt salts parenterally but in quantities much larger than 1 mg. cobalt per day.

These varying observations seem best explained on a quantitative basis. With high-level cobalt injections, enough of the element reaches the rumen to stimulate a slow response. How much cobalt reaches the rumen is not clear. The fact remains, however, that a quantity (1 mg. per day) of cobalt which when given by mouth elicits a good response will not do so when given parenterally, and thus attention was focused on the intestinal tract as the probable site of cobalt needs. This and other evidence, some admittedly viewed from hindsight, has pinpointed the probable area of the intestinal tract that is involved, namely, the stomach. That the lower tract is not primarily involved, stems from the knowledge that the bile is a significant, though not the major path, of excretion. Furthermore, Phillipson and Mitchell *(56)* found that 0.1 mg. of cobalt per day which is effective when fed, is not effective when introduced directly into the duodenum. Quantities of cobalt much higher than 0.1 mg. daily, when injected into the duodenum or abomasum, did result in positive responses in cobalt-deficient lambs and were correlated with increased cobalt concentrations in the rumen ingesta. It seems reasonable that at least in this instance, cobalt reached the rumen by antiperistalsis.

Cobalt distribution throughout the body has been studied by use of radioactive cobalt administered in various ways [Comar and Davis *(17)*, Comar *et al.*, *(18)*, Rothery *et al. (59)*, Keener *et al. (38)*, and others]. The salient findings of these studies are, that cobalt salts when fed are eliminated chiefly (80%) through the feces, with only minor amounts (0.5%) emitted via the urine; that the liver is the organ of highest cobalt concentration, and that a significant excretion occurs through the bile. When injected intravenously, cobalt rapidly disappears from the blood and 65% is excreted in the urine and about 30% in the feces. When small amounts of cobalt^{60} were injected, practically none was found in the rumen; whereas, much larger amounts permitted some accumulation in the rumen contents.

Cobalt-vitamin B_{12} relationship. Since the best evidence up to 1948 indicated a rumen-cobalt interaction, it was but a short step to think of rumen microorganisms playing a role in the metabolism of this element. A study by Gall *et al. (27)* clearly demonstrated that in cobalt-deficient lambs the rumen bacteria were reduced from a control level of 54 billion per gm. of dry material, to 30 billion; the principal types of bacteria were less than in comparable cobalt-fed lambs, and mixed cultures of bacteria obtained from cobalt-deficient lambs failed to grow beyond dilutions of 10^{-9}; whereas, cultures from positive control lambs continued to grow even at dilutions of 10^{-11}. From this preliminary study, it was evident that a shortage of cobalt significantly affected rumen bacteria — their total numbers, types present, and cultural qualities. It is unfortunate that this pilot study has not been expanded, for it would appear to be a fruitful field for research.

At this stage of progress, we set up the rather obvious working hypothesis that cobalt when fed is utilized by ruminal bacteria to produce some metabolically active compound, a shortage of which is the fundamental cause of the recognized symptoms of cobalt deficiency. A number of supplements and compounds were screened, but all were found to be inactive with the exception of commercial preparations of liver extract routinely used to treat pernicious anemia in man. Becker and Smith *(8)* reported that the daily subcutaneous injection of 15 U.S.P. units of liver extract was highly effective in curing cobalt deficiency. The same amount of liver extract was ineffective when given by mouth.

As this work was in progress, Rickes *et al. (58)* announced that vitamin B_{12} was a cobalt-containing complex. It seemed evident that this vitamin must be at least one of the metabolically active products of cobalt metabolism. However, proof that vitamin B_{12} was the primary functional form of cobalt in ruminants was slow in coming.

Merck and Company sent us a small supply (200 μg.) of crystalline B_{12}, which was immediately tested on cobalt-deficient lambs. The responses were disappointingly negative, which we now recognize were due to insufficient amounts. Since we had no information on the vitamin B_{12} requirements of any species, we were forced to use as a guess the still fragmentary information on the amount required to elicit a favorable response in pernicious anemia patients. West *(74)* had reported responses in patients given as little as 3.6 μg., intramuscularly. Other lambs were fed much larger amounts of vitamin B_{12} concentrate, but again the response was negative [Becker, Smith, and Loosli *(9)*].

Since it was impossible then to obtain more vitamin B_{12}, we decided to concentrate the active factor in liver extracts.

A commercial preparation of anti-pernicious anemia liver extract was separated into 24 fractions, using the counter-current distribution technique of Craig *(20)*. Every third fraction was tested on cobalt-deficient lambs. It was found that fractions 12 to 18, inclusively, when injected resulted in positive responses that were maximal with Fraction 15. [Smith, Koch, and Turk *(67)*]. A sample of the original liver extract when autoclaved at pH 12, a treatment that destroyed vitamin B_{12}, was inactive when tested with deficient lambs. This

TABLE 1
Response of lambs to liver fractions and vitamin B_{12}

Treatment	Change in concentrate intake (*gm/day*)	Change in total body weight (*lb.*)	Change in hemoglobin (*gm/100 ml.*)	Total vitamin B_{12} injected/lamb (*μg.*)
Liver extract, B_{12} destroyed	− 48	−3	+0.1	0
Liver extract B_{12} destroyed + crystalline B_{12}	+235	+6	+2.0	255
Crystalline B_{12}	+202	+5	+1.2	300

information, combined with the demonstration that vitamin B_{12} was also concentrated in maximum amounts in our Fractions 12-18, again strongly suggested that vitamin B_{12} was the principal product of cobalt metabolism in the body. By this time, crystalline B_{12} was available in large amounts, and it was restudied with cobalt-deficient lambs (results summarized in Table 1).

These data give ample evidence that cobalt-deficient lambs would respond to vitamin B_{12} therapy if given enough, in this instance a total of 300 μg. over a two-week period. Furthermore, it was shown that the active factor in liver extract was vitamin B_{12}. The obvious conclusion was drawn that "vitamin B_{12} is an important intermediary in cobalt metabolism in sheep." These observations were soon confirmed by Marston and Lee *(50)*, Anderson and Andrews *(2)*, and Hoekstra, Pope, and Phillips *(32)*.

It is often asked if vitamin B_{12} is the only important intermediary in cobalt metabolism. While we have no evidence now that other intermediaries are involved, future investigations may well demonstrate others. It is true that vitamin B_{12} therapy of cobalt-deficient sheep appears to be complete, inasmuch as treated lambs respond quickly in appetite and weight gains and continue to do well so long as treatment is maintained. When vitamin B_{12} administration is stopped, sheep relapse into typical cobalt deficiency. There can thus be little doubt that a cobalt deficiency in sheep is essentially a deficiency of vitamin B_{12}.

Previous observations [Becker *(6)* and Andrews and Anderson *(3)*] had suggested that vitamin B_{12} was much more effective given parenterally, rather than by mouth. Further information was desired, since by this time vitamin B_{12} feeding supplements were in general use for swine and poultry, and many wished to use this method of supplementing ruminants in cobalt-deficient areas. Kercher and Smith *(41)* found that 100 μg. of crystalline B_{12} given by mouth was ineffective in curing cobalt-deficiency; whereas, 500 μg. per day was as effective as injecting about 15 μg. per day, suggesting that only some 3% of the orally administered B_{12} was absorbed. Thus, parenterally administered vitamin B_{12} is some 35 times more effective than that given orally. Certainly, in terms of feeding practice, cobalt supplementation is the more economical method.

Progressively more detailed studies of vitamin B_{12} soon led to the realization that here was a group of related compounds, only some of which had biological activity. Brink *et al. (13)* reported that vitamin B_{12} was a cyano-cobalt complex. This led Kaczka *et al. (36)* to designate all of the B_{12} molecule, except the cyano

group, as cobalamin. This permitted the naming of specific compounds of the B_{12} family as cyano-cobalamin, hydroxo-cobalamin, etc. Some of these modifications occur naturally, whereas others can be prepared from cyano-cobalamin in simple chemical transformations in which the cyano group is replaced by a variety of anions. All members of this group seem to be readily converted to the classical vitamin B_{12} itself (cyano-cobalamin) by cyanide treatment.

One of these substitution compounds, vitamin B_{12a}, now termed hydroxo-cobalamin, was tested with cobalt-deficient lambs by Koch and Smith *(44)*. It was found to be as therapeutically effective as cyano-cobalamin.

More recently, a further series of the vitamin B_{12} group has been isolated from natural materials. Unlike the cobalamins, these compounds are not convertible to cyano-cobalamin by cyanide treatment and, furthermore, they have little or no biological activity. An excellent discussion of these cobalt-complexes has been given by Ford and Hutner *(26)*.

Kercher *(40)* tested one of these naturally occurring compounds, pseudo-B_{12} of Dion, Calkins, and Pfiffner *(21)*. When injected in amounts as high as 500 μg. into cobalt-deficient lambs, the response was negative. Similar negative results were reported for calves treated with pseudo-B_{12} by Hopper and Johnson *(33)*. The presence of pseudo–vitamin B_{12} and other B_{12}-like compounds introduces many difficulties, inasmuch as some of these compounds are microbiologically, though not biologically, active. Thus, any assay of vitamin B_{12} activity must be carefully described, since the total B_{12} values found will depend on the assay organism and other conditions of the test. According to Ford and Hutner *(26)*, only about 10% of the total B_{12} of rumen contents and feces of calves is made up of the cobalamins, the remainder consisting of B_{12}-like compounds. Such knowlege should make one very cautious in interpreting the nature of so-called "B_{12}-like fractions of tissues isolated chemically," unless the composition of such fractions is further detailed by selected microbiological, chromatographic, and spectrophotometric methods. Progress is being made in the search for microorganisms such as *Ochromonas*, that respond largely, or possibly only, to biologically active vitamin B_{12}.

Vitamin B_{12} content of tissues and ingesta. It was early shown that rumen contents and feces of ruminants are relatively rich sources of vitamin B_{12}. Hale *et al. (28)* showed a positive correlation between the cobalt intake and the B_{12} concentration of rumen contents of sheep. By the chick assay, the rumen contents of a cobalt-supplemented sheep were found to contain a minimum of 60 μg. of vitamin B_{12} per 100 g. dry material; whereas, the rumen ingesta of a cobalt-deficient sheep contained much less. Hoekstra *et al. (31)*, using *Lactobacillus leichmannii* as the assay organism, found 9 μg. of vitamin B_{12} per 100 g. dry rumen contents in cobalt-deficient sheep, compared to 130 μg. in cobalt-fed sheep. Moinuddin and Bentley *(53)*, using the same assay organism, found 100 μg. of vitamin B_{12} per 100 g. dry steer feces, and further reported that this microbiologically active B_{12} was fully active for the rat. On the other hand, only 70% of the B_{12} activity of rumen liquor was utilized by the rat.

Kercher and Smith *(43)*, also using the *L. leichmannii* assay, found an average of 104 μg. of vitamin B_{12} per 100 g. dry rumen contents of cobalt-fed animals, compared to 15 μg. in cobalt-deficient lambs. Rumen contents of cobalt-fed ruminants is thus one of the richest natural sources of this vitamin and, as might be expected, the vitamin B_{12} concentration is a function of the amount of cobalt ingested. As might be anticipated, cattle feces are also high in vitamin B_{12}. Teeri *et al (69)* observed total excretions to average about 2,200 μg. per cow per day and about 540 μg. per heifer per day. Some 99% of the excreted B_{12} was in the feces. Since cows in this study consumed 47.6 μg. per day, excretion exceeded intake some 50-fold.

The cobalt and vitamin B_{12} content of milk has been the subject of several investigations. Early, Archibald *(5)* showed that the cobalt content of cow's milk could be increased by feeding relatively massive doses (500 mg. per day) of cobalt salts. Hartman and Dryden *(29)* failed to find an increase in vitamin B_{12} in the milk of cows fed supplemental cobalt. Collins *et al. (15)* reported a range of from 2.6 to 3.8 μg. of vitamin B_{12} per liter in market milk from 23 to 57 herds. Furthermore *(13)*, there was no breed difference among Holstein, Jersey, and Guernsey cows; pasteurized milk was as rich in the vitamin as raw milk; colostrum was higher than milk. The milk of rats contains much more vitamin B_{12} than any species studied so far. In India, Sreenivasmurthy *et al. (67)* found values of from 2.7 to 9.0 for cow's milk, 2.8 to 4.0 for buffalo milk, and 0.05 to 0.16 μg. of vitamin B_{12} per liter for human milk.

Rusoff and Haq *(60)* found a range of from 1.69 to 4.12 μg. of vitamin B_{12} per liter of cow's milk, which did not change when the ration was supplemented with either chlortetracycline or vitamin B_{12}. Collins *et al. (15)* reported that the vitamin B_{12} content of goat's milk was increased the first week after parturition, when the ration was supplemented with a cobalt-containing salt mixture.

Couch *et al. (19)* reported the following values on the vitamin B_{12} concentration of the whole blood of various species: calf, 0.9, cow 0.5, sheep 0.7, rabbit

TABLE 2

Cobalt and vitamin B_{12} contents of milk

Species	Cobalt (μg/l)			Vitamin B_{12} (μg/l)		
	Mean	Range	Reference	Mean	Range	Reference
Whole milk, market	0.6	0.2–1.1	*47*	3.2	2 –25	*14, 16*
Holstein cows	—	—	—	8.0	4 –16	*4, 60*
Jersey cows	—	—	—	7.6		*4*
Sheep	—	—	—	1.4	0.07– 0.15	*16*
Goats	—	—	—	0.1	—	*16*
Buffalo	—	—	—	—	2.8 – 4.0	*67*
Human	—	—	—	0.4	0.1 – 1.5	*16*
Sow	—	—	—	1.0	0.03– 2.7	*16*
Rat	—	—	—	—	11 –95[a]	*16*
Dog	—	—	—	—	0.7 –13.0	*16*
Horse	—	—	—	0.02	—	*16*
Colostrum						
Cow	5	—	*47*	28	21 –49	*4, 16*
Goat	—	—	—	4.2	1.3 – 8.5	*16*

[a] The lower value was for rats fed an all-plant diet, and the higher value for rats fed the plaut diet plus 10 μg. vitamin B_{12} per 100 g. of diet.

10, horse 2.1, and man 0.8 mμg. per ml. Since then, many other reports have been made which will not be reviewed here, except as the blood level of ruminants varied with the cobalt intake. Hoekstra *et al. (31)* found that the vitamin B_{12} activity of the blood of sheep, in early cobalt deficiency, averaged 0.47 mμg. per ml., compared to 2.3 and 4.3 in the blood of cobalt-supplemented sheep. These observations were extended by Kercher and Smith *(42)*, who found that Western feeder lambs at purchase averaged 0.92 mμg. of vitamin B_{12} per ml. of whole blood, and that this level progressively decreased to a low of 0.03 mμg. when the lambs were fed a cobalt-deficient ration. There was a highly significant decrease in the blood vitamin B_{12} levels, considerably before the classical symptoms of anorexia, loss in body weight, and decreased hemoglobin level were observed.

In further studies, Kercher and Smith *(43)* reported blood vitamin B_{12} levels of: 0.11 mμg. per ml. for cobalt-deficient lambs, 0.09 for cobalt-deficient lambs treated by cobalt injections, and 0.62 for lambs fed 1 mg. of cobalt per day. It appears that for the first time we have a criterion, namely, blood vitamin B_{12} level, that will be of specific help in positively and quickly diagnosing a cobalt deficiency, something that can not be stated for such nonspecific symptoms as anorexia, loss in production, and an anemia.

Kercher and Smith *(43)* studied the vitamin B_{12} concentration (*L. leichmannii* assay) and total content of various segments of the gastrointestinal tract and various tissues, in lambs that were either cobalt-deficient or were treated with cobalt salts orally or parenterally. Cobalt given orally resulted in significant increases in the vitamin B_{12} concentration of the blood, kidney, pancreas, adrenal glands, the contents of the rumen-reticulum, omasum-abomasum, duodenum-jejunum, and ileum. No increases, except in the contents of the large intestine and caecum, were observed in those lambs given cobalt salts intravenously or subcutaneously. This observation is consistent with the knowledge that cobalt is excreted via the bile into the intestinal tract, and thus is available to microorganisms for the synthesis of B_{12} in the lower tract. No evidence was obtained of tissue synthesis of vitamin B_{12} from injected cobalt, contrary to the report of Monroe *et al. (54)*.

Some miscellaneous observations of Kercher and Smith *(42)* and Kercher *(40)* were, that cobalt-deficient lambs did not respond to injections of folinic acid; the vitamin B_{12} was not spared by the feeding of chlortetracycline, and that the simultaneous administration of an "intrinsic factor" concentrate, along with suboptimal amounts of vitamin B_{12}, did not give a positive response. From very limited numbers of observations, Tribe *et al. (71)* reported a delayed response in cobalt-deficient sheep treated with penicillin.

Reqirements of ruminants. The minimum cobalt requirement of sheep was closely approximated in the late thirties, chiefly by New Zealand workers. These studies are well summarized by Russell *(61)*. It was found that pastures containing 0.07 p.p.m. cobalt on a dry weight basis were on the borderline of adequacy. More recently, Marston *(48)* re-evaluated the cobalt requirement of sheep

and came to the conclusion that a total daily ingestion of 0.07 to 0.08 mg. cobalt is close to the quantity that will completely fulfill the needs for normal health. This amount is furnished by a ration containing 0.08 to 0.10 p.p.m. cobalt on a dry weight basis. Unfortunately, the requirement of cattle has not been determined so precisely. The earlier New Zealand studies suggested that cattle may have a lower requirement than sheep. It was estimated that pastures containing 0.04 p.p.m. on a dry weight basis were about borderline for cattle. In the absence of better data, it would appear that cobalt intakes adequate for sheep should also be adequate for cattle, but, obviously, final conclusions must await further studies.

The first attempts to treat cobalt-deficient lambs with vitamin B_{12} yielded negative results. It is now realized, as previously mentioned, that the amounts of the vitamin used were suboptimal. These observations led to the frequent statement that ruminants must have a high vitamin B_{12} requirement, a statement with which the authors disagree. The following table summarizes the vitamin B_{12} requirement of a number of different species. Since it is desirable to include for comparison the requirement of man, at least in terms of the pernicious anemia patient, we have selected from the literature the vitamin requirement when it is given parenterally rather than orally.

TABLE 3

Vitamin B_{12} requirement of different animals (parenteral administration)

Species	Body weight	Reported requirements	Calculated daily requirement per lb. of body wt.	Reference
			(μg)	
Rat	Av. 150 g.	0.125 μg. per day (fed thyroid)	0.37	Emerson *(25)*
Chick	Av. 120 g.	0.3 μg. weekly	0.16	Stokstad *et al. (68)*
Pig		0.6 μg/kilo body weight	0.27	Nesheim *et al. (55)*
Pig		0.26 μg/kilo body weight	0.12	Anderson and Hogan *(1)*
Sheep	Av. 60 lb.	150 μg. per 2 weeks	0.18	Smith *et al. (66)*
Human	150 lb.	1 μg. per day[a]	0.007	Bethell *et al. (12)*

[a] To relieve pernicious anemia.

Although there is variation in the reported requirements, and these data will in some cases be modified by further studies, it appears clear that the vitamin B_{12} requirement of the sheep is approximately of the same magnitude as that of other animals. The outstanding exception is man, at least insofar as remission of pernicious anemia is concerned.

By feeding a synthetic milk low in vitamin B_{12}, Draper *et al. (22)* developed a vitamin B_{12} deficiency in dairy calves characterized by cessation of growth, poor appetite and, in some cases, incoordination. Using similar techniques, Lassiter *et al. (45)* confirmed and extended these observations. In addition to growth retardation and poor appetite, they reported a white-spotted kidney condition.

Under these experimental conditions, calves required between 20 and 40 μg. vitamin B_{12} per kilogram of dry matter consumed. These figures are a little higher than those estimated to be required by the chick (15 μg. per kilo diet), reported by Stokstad *et al. (68)*, and by pigs (20 μg. per kilo diet), reported by Nesheim *et al. (55)*.

Cobalt toxicity. Excessive intakes of cobalt are toxic to many species, causing a polycythemia. In ruminants, the toxic level has fortunately proven to be very high and greatly in excess of requirements, so that overenthusiastic dosing of cattle and sheep is unlikely to cause trouble.

Ely, Dunn, and Huffman *(24)* found that cobalt fed in excess of 40 mg. daily per 100 lb. of body weight was harmful to dairy calves. The same authors later reported *(23)* that 30 mg. or more of cobalt per 100 lb. of body weight, given by intravenous injection, was also toxic. The symptoms observed were lacrimation, salivation, dyspnea, incoordination, and excessive defecation and urination. Furthermore, the injection of methionine previous to cobalt injections markedly reduced the severity of the reaction. Keener *et al. (39)* found that dairy calves were able to consume up to about 50 mg. of cobalt daily per 100 lb. body weight before untoward symptoms appeared; that is, loss of appetite, decreased water consumption, lack of muscular coordination, and some increase in erythrocyte concentration, i.e., a mild polycythemia.

Josland *(35)* drenched a few sheep with 5 mg. cobalt per kilogram of body weight and observed erratic responses ranging from a polycythemia to a mild anemia. Becker and Smith *(7)* found that, by drenching sheep with graded amounts of cobalt chloride, levels up to 160 mg. of cobalt per 100 lb. of body weight daily were tolerated with no abnormal signs, over a period of eight weeks. Levels above 160 mg. were associated with depressed appetite, loss in weight and, at very high levels, an anemia. No evidence of polycythemia was obtained.

Miscellaneous. On occasion, therapeutic value has been claimed for cobalt treatment of a number of diseases—sterility, milk fever, ketosis, brucellosis, and others. As early as 1948, Henderson *(30)* reported the successful treatment of 12 cases of ketosis with cobalt sulfate. The latest report of which the authors are aware is that of White *(75)*. So far, these claims are based on faulty or inadequate experimental techniques, especially the lack of adequate controls. If cobalt plays some role in the prevention or cure of ketosis, it remains to be shown. Similar statements can be made for its possible role in the treatment of sterility, milk fever, and brucellosis. Berman *et al. (11)* failed to find that supplementing the ration with trace-mineral elements, including cobalt, altered the course of brucellosis in dairy cattle.

The question of availability of cobalt from various sources has been inadequately studied. One would presume a ready availability of cobalt for ruminants, even from highly insoluble compounds, such as the raw mineral limonite, which was successfully used in the earliest studies. Keener *et al. (38)*, using radioactive cobalt, showed appreciable absorption of cobalt carbonate by sheep. Mittler *(52)*, using radioactive cobalt oxide, found that cobalt was well absorbed and distrib-

uted throughout the body of lambs. However, it should be recognized that these studies were more qualitative than quantitative, and much further work remains to be done.

Cobalt supplementation, as a means of practical control is, in this country, largely by direct addition to the ration. Amounts that have been recommended vary widely. For addition to the concentrate portion of the ration, the amounts most commonly added have been 2 to 4 g. of soluble salts as the sulfate, chloride, or nitrate per ton of concentrate feed. Where the carbonate is used, half this amount furnishes approximately the same amount of cobalt. The fine powdery nature of the carbonate is preferred by many feed mixers. When cobalt salts are added to a general mineral mixture, recommendations commonly have suggested 0.5 to 1.0 oz. of cobalt salts per 100 lb. of total mixture. This may be fed free-choice or added to the concentrate mixture at a level of 2%. Where such recommendations have been followed, the problem of cobalt deficiency in ruminants appears to have been eliminated.

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INFLUENCE OF RATION AND TIME OF FEEDING ON THE FREEZING POINT AND COMPOSITION OF COW'S MILK [1]

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The effects of ration composition and of feeding time on the freezing point of milk were investigated. The amount and kind of the roughage component in the ration did not affect the freezing point of milk. When roughage was offered at 10 a.m. and at 3 p.m., the cows consumed more water between the morning and evening milking than for either the preceding or succeeding 12-hr. periods, and the evening's milk had a lower freezing point. When roughage was offered at 6 a.m. and 6 p.m. the differences in water consumption and freezing point were reduced. Cows fed at uniform intervals consumed more feed and produced more milk than those fed at irregular intervals.
Editor.

The freezing point of cow's milk is of scientific and practical interest. It is the least variable property of milk, but the fact that it does vary within relatively narrow limits, is of considerable importance. The variations observed by early workers have been reviewed *(16)*. Critical studies on the effect of diet and management on the freezing point are few. Shipe *et al. (16)* reported that the freezing point of milk from cows on pasture was lower than when the cows were on an all-hay ration. The freezing point of milk produced on the hay ration was lower than when a portion of the hay was replaced with grain. They further noted that the freezing point for the evening milk was lower than that for the morning milk on the rations containing hay; whereas, for cows on pasture the reverse was true. In an effort to determine the cause for the observations noted above, two additional experiments were conducted. The first was designed to determine the effect of the ratio of hay to grain, and the second to determine the effect of ration, as well as time of feeding, on the freezing point of milk. In the second experiment, milk composition was also studied, to determine whether it might provide an explanation for some of the freezing point variations observed.

EXPERIMENTAL PROCEDURE

Experiment I. Four purebred Holstein-Friesian cows that had passed their peak of lactation and had not been rebred were selected for the study. They were allowed a U. S. No. 3 alfalfa-light timothy mixed hay, free choice, as the sole feed during a 6- to 10-wk. preliminary period. They were then assigned at

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random to a 4 × 4 Latin-square design and received the rations shown in Table 2. The grain mixture consisted of ground corn, 70% ; corn distillers dried grains, 28% ; iodized salt, 1%, and dicalcium phosphate, 1%. Substitutions were made on a weight basis, with the total pounds of feed consumed being reduced by 5% at the start of each succeeding period. Cows were fed hay at 10 A.M. and 3 P.M. and grain at 5 A.M. and 3 P.M. They were milked from 5 to 6 A.M. and from 4 to 5 P.M. Periods were of 5 wk. duration and milk samples were taken for freezing-point determinations from the morning and evening milkings, for two days during each of the last 4 wk. of each period. The modified cryoscope *(15)* was used for these determinations. The cows were housed in adjacent stanchions, equipped with water cups and bedded with shavings or sawdust. Statistical analysis of the data was made, using the analysis of variance technique, Snedecor *(17)*, and the multiple range test of Duncan *(4)*.

Experiment II. Four purebred Holstein-Friesian cows that had recently passed their peak of lactation and had not been rebred were selected for the study. They were fed a mixed hay free choice and approximately 18 lb. daily of a 16% commercial dairy feed for a 3- to 4-wk. preliminary period, to adjust feed intakes and establish levels of production. During the last week of the preliminary period the experimental roughage, consisting of a mixed alfalfa timothy hay, was offered.

The rations were as follows: *(1)* Hay plus a 16% commercial dairy feed; *(2)* equal parts of beet pulp and citrus pulp, plus a 24% total protein commercial dairy feed. The beet-citrus pulp mixture replaced all of the hay on an estimated net energy basis *(11)*, at a rate of 0.56 lb. for each lb. of hay. The level of beet-citrus pulp feeding was based on the average daily hay consumption during the most recent 2 wk. of hay feeding. The high protein feed was used to insure adequate digestible protein intake on the beet-citrus pulp ration. Animals were fed all the feed they would clean up. In all cases the "roughage" component constituted 60 to 67% of the total ration on a weight basis.

The two cows assigned to each of the diets were further divided as to feeding interval. One of each pair received its roughage component at 10 A.M. and 3 P.M. (irregular feeding interval) ; whereas, the other received it at 6 A.M. and 6 P.M. (uniform feeding interval). Grain was fed to all animals at 3 P.M. and 5 A.M. It was anticipated that cows on the uniform schedule would consume feed at a fairly uniform rate during a 24-hr. period, and that cows on the 10 A.M. and 3 P.M. schedule would consume more hay between the A.M. and P.M. milking. These treatments will be referred to as BC-U (beet-citrus uniform feeding interval), BC-I (beet-citrus irregular feeding interval), H-U (hay uniform feeding interval), and H-I (hay irregular feeding interval). Cows were milked with the herd from 5 to 6 A.M. and from 4 to 5 P.M. They were housed in adjacent stanchions equipped with metered water cups and bedded with shavings or sawdust.

The experimental plan consisted of a 2 × 2 factorial arrangement of a 4 × 4 Latin-square design, consisting of periods of 4 wk. duration. The first week was considered as transitional, and the data from the last 3 wk. were averaged for comparison. Cows were assigned at random to the initial treatment.

Milk was weighed to the nearest one-tenth of a pound at each milking. The beet and citrus pulp and grain were completely consumed, with the exception of two cows who refused some grain during the last three days of one period. Hay refusals were about 5 to 10% and were recorded daily at 10 A.M. and 6 A.M. for the irregular and uniform feeding groups, respectively. Water intakes were recorded immediately after each milking. One-pint samples of milk were collected from each morning and evening milking on two consecutive days, during each of the last 3 wk. of each period. Freezing point determinations were always made within 5 hr. after milking, as described above, the milk being held at 35 to 38° F. until tested. The samples were tested for fat content by the Babcock method *(2)*, and for total solids using the Mojonnier method *(10)*. In the 4th wk. of each period the milk was also analyzed for chlorides by the Sharp and Struble titration procedure *(10)*, and for lactose, using a micro-method *(5)*. The freezing point determinations and the analyses described above were made in duplicate and averages reported. Body-weight and barrel-circumference measurements were made the last three days of each period.

One cow died at the beginning of the third period and the missing data were calculated, using the "missing plot" technique *(17)*. Differences between treatments were evaluated by an analysis of variance *(17)*. When statistically significant, the treatment mean square was divided into its three individual degrees of freedom, viz., roughage, feeding interval, and interaction. As the missing plot calculations introduced an upward bias to the F ratios calculated in the usual manner, these ratios were adjusted for this bias, using a method suggested by Henderson *(6)*.

RESULTS AND DISCUSSION

The chemical composition of some of the feeds *(1)* are shown (Table 1).

Experiment I. The results of the freezing-point measurements are shown (Table 2). Data on ration digestibility, feed intakes, and milk and fat production for these cows will be combined with other similar data, and reported in a subsequent paper.

TABLE 1

Chemical composition of some of the feeds used in Experiments I and II

	H_2O	Protein	Ether extract	Crude fiber	Ash	NFE
	(%)					
			Experiment I			
Hay[a]	8.0	13.5	1.9	28.4	5.8	42.4
Grain	7.4	14.5	3.7	5.5	2.8	66.1
			Experiment II			
Hay[b]	8.2	8.4	1.9	33.3	5.3	42.9

[a] U. S. No. 3 alfalfa-light timothy mixed hay. Hay plants consisted of: alfalfa 67.9%, clover 6.9%, timothy 25.2%, color 69%, foreign material 14.4%.

[b] U. S. sample-grade mixed hay. Hay plants consisted of: timothy 42.4%, alfalfa 37.9%, blue grass 8.5%, quack grass 5.7%, clover 5.5%, color 42%, foreign material 30%.

TABLE 2

Freezing points of milk from cows fed different ratios of hay to grain[a]

Ration	Freezing point			
	Daily average[b]	A.M.	P.M.	A.M.–P.M.
	(° C.)			
1—100% hay	−0.541	−0.534	−0.550	+0.016[c]
2— 75% hay, 25% grain	−0.543	−0.538	−0.551	+0.013[d]
3— 50% hay, 50% grain	−0.544	−0.540	−0.550	+0.010
4— 35% hay, 65% grain	−0.543	−0.538	−0.548	+0.010

[a] The A.M. and P.M. freezing point values represent the averages of the observations made on the samples taken during the last 4 wk. of each period, *i.e.*, eight A.M. and eight P.M. samples.

[b] The daily average freezing point values are weighted for A.M. and P.M. milk production.

[c] Significantly greater than 3 and 4 ($P < 0.01$) and 2 ($P < 0.10$).

[d] Significantly greater than 3 and 4 ($P < 0.10$).

The average daily freezing point represents an average of the morning and evening samples weighted for production. The differences between rations were not significant, which does not confirm the observations previously reported *(16)*. For all cows on all rations, the P.M. freezing point was lower than the A.M. freezing point. Furthermore, the differences between the A.M. and P.M. freezing points became smaller as the proportion of hay decreased.

These results, coupled with the observations of Shipe *et al. (16)*, suggested an interesting hypothesis. The cows in the above study, and also the barn-fed cows reported earlier *(16)*, were fed under conditions in which the cows were offered all of their hay between 10 A.M. and 3 P.M. Grain was given just before milking at 5 A.M. and 3 P.M. The fact that the P.M. milk had a lower freezing point that the A.M. milk, suggested that the periods of intensive feed consumption were conducive to a lowered freezing point. This theory received further support by the observation *(16)* that cows on pasture gave milk with a lower freezing point in the morning, presumably following periods of more intensive pasture consumption during the night. The data in the above study also suggested that the A.M.–P.M. differences might be influenced by the amount of grain feeding, with a higher level of grain feeding reducing this difference. In light of these observations, the second experiment was conducted.

Experiment II. Morning and evening comparisons. The average freezing points for morning and evening samples of milk from individual cows are shown (Table 3), as an indication of the variability noted for the experimental animals. The range of freezing points for all of the individual samples taken during the entire experiment was from −0.528 to −0.563° C.

Table 4 summarizes the effect of the treatments on the water intake of the cows, as well as the freezing point, lactose, chloride, solids-not-fat, and fat content of the morning and evening milk samples. The data have been presented to show the average values obtained for each treatment. In addition, the difference between the morning and evening results (A.M.–P.M.) have been summarized for all observations made for each feeding interval and each type of roughage.

TABLE 3

The effect of ration and management of cows on the freezing point of morning and evening milk[a]

Cow No.	Beet-citrus pulp				Hay			
	Uniform		Irregular		Uniform		Irregular	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
	(° C.)							
130	−0.537	−0.538	−0.539	−0.542	−0.535	−0.532	−0.539	−0.545
149	−0.532	−0.532	−0.538	−0.544	−0.535[b]	−0.532[b]	−0.533[b]	−0.540[b]
170	−0.536	−0.538	−0.538	−0.545	−0.544	−0.534	−0.538	−0.544
196	−0.538	−0.541	−0.535	−0.551	−0.539	−0.538	−0.536	−0.547
Av.	−0.536	−0.537	−0.538	−0.546	−0.538	−0.534	−0.536	−0.544
High[c]	−0.528	−0.529	−0.529	−0.531	−0.531	−0.528	−0.530	−0.532
Low[c]	−0.543	−0.547	−0.543	−0.563	−0.549	−0.541	−0.543	−0.552

[a] The individual values represent the average freezing point of the samples taken during the last 3 wk. of each period, *i.e.*, six A.M. and six P.M. samples.

[b] These values were determined by use of the "missing plot" formula for a Latin-square.

[c] These values represent the highest and lowest values of all of the individual samples measured.

The data show that the difference between the freezing point of morning and evening milk was significantly larger ($P < 0.01$) for cows being fed at irregular intervals than for those being fed at uniform intervals, with the evening milk having the lower absolute freezing point. The difference between roughages was not significant, which is contrary to what one might have expected from Experiment I. Hay provided about 65% of the total feed in Experiment II; whereas, in Experiment I, the greatest A.M.–P.M. differences were on diets containing more than 75% hay. It is possible that if a higher level and better quality of hay had been fed in Experiment II, the contrast between the hay and the beet-citrus rations might have been more pronounced.

The differences between the morning and evening values for lactose and chlorides were not significant, and showed no relationship to the observed changes in freezing point. This lack of correlation is not surprising, since the changes in freezing point could be effected by solute changes in the milk too small to be accurately detected by the chemical procedures employed. It is also possible that the concentration of other components not measured changed sufficiently to account for the freezing-point alterations. The differences between the morning and evening values of solids-not-fat and fat were not statistically significant.

Significantly more water was consumed between the morning and evening milking by cows receiving their roughage during this interval, than for cows which were fed roughage immediately after each milking. This fact is evidenced by a greater A.M.–P.M. difference in water consumption for the irregular feeding interval, compared with the uniform feeding interval (Table 4). The increased consumption of water corresponds to the time when the roughage was offered and was reflected by a lower freezing point of the P.M. milk. The lower freezing point of milk, following a period of rapid feed and water ingestion, seems readily explainable from an examination of the literature.

TABLE 4

The effects of ration and management of cows on the morning and evening water intake of the animals, the freezing point and the lactose, chloride, solids-not-fat, and fat content of the milk[a]

Feeding interval	Roughage	Freezing point	Lactose	Chlorides	S.N.F.	Fat	Water intake
		(°C.)			(%)		(lb.)
Morning							
Uniform	Beet-citrus	−0.536	4.46	0.15	8.21	3.4	67.8
	Hay	−0.538	4.49	0.16	8.17	3.8	83.9
Irregular	Beet-citrus	−0.538	4.54	0.15	8.23	3.3	40.8
	Hay	−0.536	4.43	0.16	8.04	3.5	57.8
Average		−0.537	4.48	0.155	8.142	3.5	62.6
Evening							
Uniform	Beet-citrus	−0.537	4.54	0.15	8.47	3.3	55.9
	Hay	−0.534	4.58	0.16	8.12	3.4	78.0
Irregular	Beet-citrus	−0.546	4.42	0.15	8.42	3.3	71.0
	Hay	−0.544	4.49	0.16	8.34	3.3	95.2
Average		−0.5402	4.51	0.155	8.337	3.32	75.0
Morning–evening difference							
Uniform		−0.001	−0.085	0	−0.105	+0.25	+ 8.9
Irregular		+0.008**	+0.030	0	−0.245	+0.10	−33.3**
	Beet-citrus	+0.004	+0.020	0	−0.225	+0.05	− 8.6
	Hay	+0.002	−0.075	0	−0.125	+0.30	−15.8

** Significantly different ($P < 0.01$) than the uniform value.

[a] The freezing point, solids-not-fat, and fat values represent the averages of the observations made on the milk samples taken from the four cows during the last 3 wk. of each period; *i.e.*, six A.M. and six P.M. samples. The lactose and chloride values represent the averages of the observations made on the samples taken during the last week of each period, *i.e.*, two A.M. and two P.M. samples. The water intake values represent the averages of the observations made in all the days of the last 3 wk. of each period, the A.M. water intake being the water consumed between the evening and morning milking, and the P.M. water intake that consumed between morning and evening milking.

Early work by Van der Laan in Germany *(19)* showed that the freezing point of cow's blood could be raised by rapid water ingestion and lowered by feeding sodium sulfate. He further demonstrated that changes in the milk freezing point followed closely the changes in the freezing point of blood. A recent British publication *(12)* has reported that the freezing point of milk can be markedly increased (0.030 to 0.040° C.) by allowing cows free access to water following periods of water restriction. Presumably the "watering" of the milk reflected the watering of the blood. In the present study, however, increased water and feed consumption has resulted in a more concentrated milk. It appears that the diluting effect of water consumption, as noted in the above report, has been counteracted by the increase in feed consumption. It is well known *(1, 13, 14)* that the rate of rumen fermentation increases perceptibly following feeding, giving rise to an increase in metabolites in the blood stream. This increase in blood metabolites could, in turn, result in the synthesis of milk of a sufficiently more concentrated composition, to be detected by the cryoscopic technique used here. The fact that the difference was small between the morning and evening freezing point of milk, from animals fed at regular 12-hr. intervals, and consuming similar amounts of water during these periods, tends to confirm this hypothesis.

TABLE 5

The effects of ration and management on the daily milk composition and production, and other observations on lactating dairy cows

Treatment		Composition[a]					Production[b]		Other observations[c]				
Feeding interval	Roughage	Freezing point	Lactose	Chloride	SNF	Fat	Milk	FCM	Concentrate intake	Roughage intake	Water intake	Body wt.	Barrel circum.
		(°C.)	(%)				(lb.)						(in.)
Uniform	Beet-citrus	−0.536	4.50	0.15	8.29	3.35	49.1	44.4	16.5	18.8	123.2	1317	94.7
	Hay	−0.536	4.55	0.15	8.15	3.55	41.4	38.7	16.7	31.7	155.4	1362	98.6
Irregular	Beet-citrus	−0.541	4.56	0.16	8.30	3.27	46.7	41.2	15.9	17.4	111.6	1277	94.0
	Hay	−0.540	4.46	0.15	8.26	3.42	39.1	35.7	16.6	29.7	150.8	1356	98.0
Uniform		−0.536	4.52	0.15	8.22	3.45	45.3*	41.5[d]	—	—	139.3	1340[d]	96.7
Irregular		−0.540*	4.51	0.15	8.28	3.35	42.9	38.5	—	—	131.2	1316	96.0
	Beet-citrus	−0.538	4.52	0.15	8.30	3.31	47.9**	42.8*	—	—	117.4	1297	94.3
	Hay	−0.528	4.50	0.15	8.20	3.49	40.3	37.2	—	—	153.1**	1359**	98.3**

* Significantly different from either the other feeding interval or roughage ($P < 0.05$).

** Significantly different from either the other feeding interval or roughage ($P < 0.01$).

[a] The freezing point, solids-not-fat, and fat values represent the averages of these observations made on the morning and evening milk from the four cows, weighted for the milk produced at each milking for the six days of sampling in the last 3 wk. of each period; and the lactose and chloride values for two days of sampling in the last week of each period.

[b] These values represent the daily average milk and fat-corrected milk produced per animal, on all the days of the last 3 wk. of each period, *i.e.*, 21 days.

[c] The feed and water intake values represent the daily average amount consumed per animal, on all the days of the last 3 wk. of each period, *i.e.*, 21 days. Body-weight and barrel-circumference measurements represent an average of three daily measurements made on the last three days of each period.

[d] Significantly different from the irregular group ($P < 0.10$).

Daily average comparison. A comparison of the influence of treatment on the composition of the milk, as well as certain measurements made on the cows, is shown (Table 5). The differences between treatment with respect to freezing point, lactose, chloride, solids-not-fat, or fat percentage were not statistically significant. The average fat percentages do suggest, however, that the beet-citrus ration resulted in a lower fat test, which is in agreement with the early findings of Loosli *et al. (9)* and Balch *et al. (3)*. In view of the results of recent research [Balch *et al. (3)* and Krienke *et al. (7)*], it is probable that the feeding periods were too short to show the maximum effect of low roughage on the fat content of the milk.

Cows receiving the beet-citrus ration produced significantly more milk than those on the hay rations ($P < 0.01$). This difference is probably a reflection of a higher energy consumption when the beet-citrus ration was fed. Although the replacement of hay with the beet-citrus pulp was intended to be on an isocaloric basis, an overestimation of the net energy content of the hay could readily account for the above effect. This problem has been discussed by Loosli *et al. (8)*. If a factor of 75, 70, and 40 therms per 100 lb. of beet-citrus pulp, concentrate and hay, respectively, are applied to the feed intake data, the daily estimated net energy intake for the four treatments in therms are BC-U 25.7, BC-I 24.1, H-U 24.4, and H-I 23.5. These values are in the same general order as the amount of FCM produced by these treatments.

There was a significantly larger amount of milk ($P < 0.05$) and FCM ($P < 0.10$) produced during periods of uniform feeding. The amount of increase is roughly comparable to the increase in feed consumption during these periods. To what extent this observation may be of practical importance can not be determined from this study.

It is interesting that all cows lost weight on the beet-citrus ration. The drop in weight is probably a loss of fill, since the barrel circumference and average daily water consumption were significantly less for cows on the hay-free rations (Table 5). The reduced water intake on a low-hay ration has been reported previously *(16)*.

TABLE 6

Error variance and F ratios of all observations which were statistically significant ($P < 0.10$), after the treatment variances had been corrected for the upward bias as a result of the missing plot calculations[a]

Observation	Error variance	F ratio[b]	
		Roughage	Feeding interval
Freezing point (A.M.–P.M.)	9.72	——	27.8
Water intake (A.M.–P.M.)	2.92	——	33.9
Water intake (daily av.)	1.65	32.2	——
Freezing point (daily av.)	4.0	——	14.2
Milk production (daily av.)	1.8	88.3	10.1
FCM (daily av.)	4.7	16.4	6.5
Barrel circum. (daily av.)	1.82	22.5	——
Body weight (daily av.)	255.3	28.5	6.7

[a] In no instance was the interaction (roughage × feeding interval) significant.
[b] F ratio required for significance; 1% 21.20, 5% 7.71, 10% 4.54.

The error variance and F ratios of all observations which were statistically significant ($P < 0.10$) have been summarized (Table 6).

SUMMARY

Two experiments were conducted with Holstein-Friesian cows, using a Latin-square design to determine the influence of several rations and the time of feeding on the freezing point of milk.

In one experiment, hay constituted 100%, 75%, 50%, or 35% of the total ration by weight. The remainder of the ration was composed of a simple grain mixture of total protein content similar to that of the hay. There were no significant differences between the daily average freezing points of milk produced by cows on these four rations. The freezing point of the evening milk was lower than that of the morning milk.

In a second study, a basal ration consisting of 65% hay and 35% grain was compared with one in which the hay was replaced on an estimated net energy basis, by a mixture of equal parts of beet pulp and citrus pulp. There was no difference in the daily average freezing point of milk due to these rations.

When the roughage component in this latter study was offered at 10 A.M. and 3 P.M., there was a significantly greater water intake between the morning and evening milking than for the previous or succeeding 12-hr. period. Further, the evening milk had a lower freezing point than the morning milk. When the roughage was offered at 6 A.M. and 6 P.M., the differences of freezing point and in water consumption were reduced. It is suggested that the rapid absorption of nutrients following a rapid feed intake results in the secretion of milk of a slightly more concentrated composition. Analysis of the milk for chlorides, lactose, fat, and solids-not-fat did not reveal any specific variations which might explain the observed changes in the freezing point. It was pointed out, however, that the precision of the chemical assays is much less than the cryoscopic technique employed, and that the concentration of other components which were not measured in the experiment may have changed enough to account for the freezing-point alterations.

Significantly more milk and FCM was produced by cows on the beet-citrus ration than by those on hay. Cows being fed at uniform intervals consumed slightly more feed and produced more milk and FCM than those fed at irregular intervals.

Water intakes and barrel-circumference measurements were larger for cows on the hay rations, which appears to explain the larger live weight of these cows compared to the values when they were receiving the beet-citrus ration.

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EFFECT OF FEEDING STABILIZED BEEF TALLOW ON MILK PRODUCTION AND COMPOSITION[1]

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Rendered beef tallow was fed to lactating cows and its effect on blood serum lipids, milk composition, and production was studied. Blood serum lipid was increased by feeding a concentrate mixture containing 7% beef tallow. Animals on the high-fat ration produced more milk than those on the control ration. *Editor.*

Several factors, including the development of synthetic detergents, have changed the utilization of animal fat by-products. Reduced demand for these materials has created disposal problems for the meat-packing industry. Some use has been made of rendered animal fats in rations, for both ruminant and nonruminant animals *(5, 7, 11, 12, 13, 15)*. There have been studies reported relating to the use of various types and amounts of vegetable fats and oils in rations for dairy cows *(4, 6, 18)*. Some reports[3] have been made concerning fats of animal origin in rations of dairy cows and calves *(8, 9, 10, 17, 19)*.

This study was conducted to determine the value of rendered beef tallow in rations for lactating dairy cows, and its effect on milk production and composition.

EXPERIMENTAL PROCEDURE

Feeding regime. A preliminary study *(2)* showed that a concentrate ration with 5% added beef tallow was readily consumed by dairy cows. On the other hand, a ration with 8% added tallow was refused occasionally. In this study, therefore, a ration containing 7% added fat was selected. The tallow was stabilized with 0.1% butylated hydroxy toluene. For comparison, a regular concentrate ration served as the control, and a ration with added stabilized beef tallow served as the experimental ration. The composition of the control and high fat concentrate rations is shown (Table 1).

In the trials, control and experimental groups consisting of nine each Guernsey and Holstein cows were used. In each case, the two groups were balanced as closely as possible according to breed, age, size, milk and fat production, and length of current lactation, with all animals having calved at least eight weeks prior to the start of the trials.

Two double reversal feeding trials with 28-day periods were used, one in summer and one in winter. The concentrate mixture with added beef tallow had approximately 10% more TDN and DP than the control ration (Table 1). In feeding the control ration, therefore, an amount was fed which was equivalent

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[2] Resigned, August 15, 1956.

[3] The reports, *(9)* and *(10)*, appeared while this manuscript was being prepared for publication.

TABLE 1
Percentage composition of the concentrate rations

Ingredient	Control		High fat
Rolled barley	19		12
Dried citrus pulp	20		20
Wheat bran	20		23
Rolled milo	18.1		3
Cottonseed meal	11.4		18
Molasses	9.5		10
Salt	1		1
Bonemeal	1		1
Animal fat	—		7
Total	100.0		100.0
Total ether extract	2.55		9.35
TDN[a]	71.4	(78.5)[b]	79.1
Total crude protein[a]	10.9	(12.1)[b]	12.1

[a] Calculated from values given by Morrison *(14)*.
[b] 110% of the calculated amount.

to 10% more than the basic rate given below. A gradual conversion was made from one concentrate to the other, with a change-over period of seven days between each experimental period. Concentrates were fed to Guernsey cows at the basic rate of 1 lb. for each 4 lb. milk produced, and the Holstein cows at the rate of 1 lb. to 5 lb. milk produced. The 28-day periods were selected in order, *(a)* to complete the trials during the season specified, and *(b)* to maintain the animals near the middle of their lactation periods. Weekly adjustments were made in the concentrate feeding rate according to changes in milk production. Each group was fed roughage consisting of 1½ lb. of good quality alfalfa hay and 3 lb. of hegari silage per 100 lb. body weight.

Body weight, milk production, and blood serum lipids. Body weight data was compiled by averaging the weights taken on three consecutive days, at the beginning of each experimental period and at the end of the last period. Daily milk weights were used to determine milk production. Blood serum lipid determinations were made weekly on samples of venous blood from the jugular vein. An analytical procedure was applied similar to that used in the ether extract method of estimating fat in fluid dairy products.

Milk composition analyses. The Reichert-Meissl, Polenski, and iodine values of milk fat were determined at the end of each experimental period by A.O.A.C. methods *(1)*. Per cent butterfat was determined by the Babcock method once a week on a composite sample of the morning and evening milkings. Specific gravity was determined by the lactometer at 60° F.

RESULTS AND DISCUSSION

There were no significant differences in the results obtained in the two trials. Data from only one trial will be presented and discussed. All animals readily consumed the concentrate mixture with 7% added fat. No digestive or other physiological disturbances were noticed.

Blood serum lipids, body weight, milk production, and milk composition of animals on the control and high-fat rations are given (Table 2). Analysis of

TABLE 2

Blood serum lipids, body weight, milk production, and milk composition of animals on control and high-fat rations

Item	Control Adjusted mean	High fat Adjusted mean	95% confidence interval for true difference
Blood serum lipids *(mg/100 ml.)*	469.1	605.7	136.6 ± 27.0[a]
Body weight *(lb.)*	1,207.8	1,214.0	6.2 ± 10.7
Milk production *(lb. 4% F.C.M/cow/day)*	32.0	35.3	3.3 ± 1.9[a]
Milk composition			
Butterfat *(%)*	4.56	4.65	0.09 ± 0.10
Iodine No.	33.8	36.4	2.6 ± 1.3[a]
Reichert-Meissl No.	29.9	31.1	1.2 ± 1.8
Polenski No.	2.98	2.34	0.64 ± 0.32[a]
Specific gravity	1.0341	1.0337	0.0004 ± 0.0005

[a] Significant.

variance was by the method of Snedecor *(16)*. Feeding 7% added animal fat significantly increased the blood serum lipids. This is in agreement with the findings of Bender and Maynard *(3)*, who reported a close relationship between total blood serum lipids and changes in the fat intake. There were no differences in the mean body weights of the animals on the two rations.

Under the conditions of this experiment, animals on the animal fat concentrate mixture produced significantly more milk than those on the control ration. This does not agree with the recent report by Horton *et al. (9)*. The differences might be due to three factors: First, "hydrogenated tallow" was used in the Illinois study; whereas, a nonhydrogenated product was used in these trials. Secondly, different experimental designs were involved in the two studies. The experimental design used by Horton may not adjust for possible variations in lactation curves; whereas, the change-over or switchback design used in this study tends to compensate for these variations *(16)*. Thirdly, different roughage-feeding regimes were used in the two studies.

There were no differences in the per cent butterfat, Reichert-Meissl number or specific gravity of the milk produced on the two rations. Milk fat produced by animals on the high-fat ration had a slightly higher iodine number than that produced on the control ration. Conversely, the Polenski number was slightly higher on the control ration than on the high-fat ration. The values for the Reichert-Meissl and Polenski numbers agree closely with those reported recently for milk produced in the region *(20)*.

SUMMARY

Stabilized beef tallow was fed to lactating dairy cows and its effect on blood serum lipids concentration, milk production, and milk composition was observed.

The concentration of blood serum lipids was significantly increased by feeding a concentrate mixture with 7% added beef tallow.

Animals on the high-fat ration produced significantly more milk than those on the control ration.

There were only minor differences in the composition of the milk produced by animals on the two rations.

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EFFECTS OF FEEDING AUREOMYCIN TO DAIRY CALVES[1]

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This study was initiated to determine the effects of feeding crystalline aureomycin hydrochloride in milk from birth to 60 days, and in the concentrate mixture from 61 to 120 days, and of removing it at 61 and at 121 days. Calves fed 45 mg. daily per 100 lb. weight from birth to 60 days, gained 50 lb. as compared to 45.8 lb. for the controls. Those continued from 61 to 120 days on alfalfa hay plus concentrate containing 50 p.p.m. of the antibiotic, gained 76.1 lb. as compared to 66.4 lb. for the controls. Weight gain and hay consumption were significantly lowered when aureomycin was removed from six calves after 61 days. Responses of animals to removal of aureomycin varied from excellent gains in weight, feed intake, and appearance to conditions of anorexia, reduced growth, lachrymation, diarrhea, rough coat, and thick, scaly hide. *Editor.*

Most experiments have shown that young calves fed rations containing aureomycin (chlortetracycline) made faster weight gains than those consuming unsupplemented rations, but some workers have not obtained significant weight increases by feeding this antibiotic. Reports on the effects of administering aureomycin to dairy cattle have been reviewed by Reid, Warner, and Loosli *(35)* and by Lassiter *(21)*, with other publications *(5, 15, 18, 20, 28, 32, 33, 37, 39)* appearing subsequently.

The action of aureomycin in improving weight gains and health of cattle has not been clarified. Possible modes suggested include, stimulation of appetite *(16, 26)*, causing an increase in output of growth hormone by the pituitary *(36)*, alteration of energy metabolism *(13)*, reducing the incidence of diarrhea *(2)*, and changing the physiology of intestinal bacteria so that they become less detrimental to the host *(33)*. Many workers have reported beneficial effects on growth where the incidence of calfhood diseases, particularly scours, was lowered by aureomycin feeding *(2, 3, 4, 8, 14, 20, 24, 27, 28)*; whereas, others have obtained significantly higher gains from its use where diarrhea was not a problem *(7, 10, 18, 19, 23)*. This observation does not preclude the possibility that beneficial results may accrue from control of subclinical or undiagnosed infections.

Weight gain rates reported for control and for aureomycin-fed calves by different investigators have varied considerably, due to age, breed, sex, feeding and management regimes, and environment. In many of the studies where positive results were obtained from feeding aureomycin to calves, the average growth rate of the control groups was below the Missouri normal growth standard *(34)*. Although significantly larger gains have been reported for animals fed aureomycin, in experiments where the control groups made normal growth or above *(6, 9, 30, 32)*, some workers did not find significant differences in

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other studies *(15, 22, 23, 39)* where average gains of the control groups were at similar rates. Hogue *et al. (14)* also reported no advantage from feeding aureomycin beyond seven weeks, and Gaunya *et al. (11)* found no significant growth increase resulting from the incorporation of 0.25% of Aurofac 2A in the concentrate of calves eight through 91 days of age. In cows fed aureomycin, Lodge, Miles, and Jacobson *(25)* recently reported the emergence of strains of *Pseudomonas* and *Monilia* that can inactivate aureomycin, and suggested that this may explain the lack of positive differences between some aureomycin-fed and control animals.

In experiments where observations were reported following removal of aureomycin from the diet of calves, the results have been variable. When aureomycin feeding was discontinued at eight weeks, no adverse effects were noted on growth rate through 12 weeks of age *(7)*. Ellsworth *et al. (9)* observed that following termination of aureomycin feeding at 100 days, growth first slowed and then accelerated above that of the controls, so that gains were the same for both groups at 160 days. Jacobson, Kaffetzakis, and Murley *(17)* indicated that in most instances there were no adverse effects resulting from the removal or initial introduction of aureomycin into the diet at 116 days of age. Later, it was reported *(16)* that following discontinuation of aureomycin feeding at 116 days gain rates did not drop off, but that there was a slight dip in the growth curve at 193 days of age. When aureomycin was removed from the diet at seven weeks, Bartley *et al. (3)* found that the growth rate slowed, but at 12 wk. the animals were in better condition than the controls. Following discontinuation of aureomycin feeding at 12 and 13 wk. of age, Hibbs, Conrad, and Pounden *(13)* observed a decline in growth rate of the calves, so that their weight was approximately equivalent to that of the control group at 20 wk. of age. McGilliard *et al. (29)* reported a small decline in growth rate after termination of aureomycin feeding at 35 days of age, so that both the control and experimental groups weighed approximately the same at 112 days. Inoculation of some of the aureomycin-fed calves with cud on the 36th and 41st day did not affect body weight gains at 16 wk. of age. After removal of aureomycin from the diet at 96 days of age, Edgerly *(8)* noted that normal gains were continued for about one week, followed by a two- to three-week adjustment period, during which there was a tendency for undue laxation among the animals. After four weeks, the animals were back to normal and making gains equal to those of the controls.

This study was undertaken to determine: *(a)* the effect of feeding crystalline aureomycin hydrochloride in the milk from birth through 60 days of age and in the concentrate mixture from 61 through 120 days, under conditions where the control animals would make normal gains or above, and *(b)* the effects of removing aureomycin from the diet at 61 and at 121 days of age.

EXPERIMENTAL PROCEDURE

Thirty new-born Jersey females and two males were divided into two comparable groups. Each calf was fed its dam's milk for three days, whole milk

four through 21 days, and reconstituted skimmilk 22 through 60 days of age. Cod-liver oil containing about 100,000 I.U. of vitamin A was mixed in the milk for each calf three times weekly. Milks were fed from nipple-pails twice daily, at the rate of 4.5% of body weight per feeding. Good quality chopped alfalfa hay was fed ad libitum and a concentrate mixture containing ground oats, 25%; ground corn, 30%; wheat bran, 10%; nonfat dry milk solids, 10%; peanut meal, 13%; salt, 1%; and bone meal, 1%, was fed free choice up to a maximum consumption of 4 lb. daily per animal. Records were kept of daily feed consumption, body weight, and general condition.

In the first experiment, calves in the experimental group were fed crystalline aureomycin hydrochloride dispersed in the morning and evening milk allowances at a daily rate of 45 mg. per 100 lb. of body weight. Their pair-mates in the control group did not receive aureomycin. At 61 days of age, aureomycin feeding was discontinued with six females and these, along with their pair-mates, were continued through 90 days of age on concentrate plus hay.

In Experiment II, seven calves from the control group of Experiment I were continued from 61 through 120 days, and six of these seven from 121 through 150 days of age on concentrate mixture and chopped alfalfa hay. Their pair-mates from the experimental group of Experiment I were fed similarly, except that 50 p.p.m. of crystalline aureomycin hydrochloride was incorporated into the concentrate mixture fed from 61 through 120 days of age.

RESULTS AND DISCUSSION

Gains from birth through 60 days of age averaged 45.8 lb. for the control group and 50.0 lb. for the aureomycin-fed animals (Table 1). This difference in growth rate was not statistically significant *(38)*. Gains of the control group averaged 124% of the Missouri normal growth standard *(34)*, and those of the experimental group were 135% of this standard.

TABLE 1

Summary of average initial body weights, gains, and feed intakes of calves in control and experimental groups, from birth through 60-day aureomycin supplementation period, and the 61- through 90-day post-aureomycin feeding period

	Control group	Experimental group
	Aureomycin feeding period, birth through 60 days	
No. of calves	16	16
Av. birth weight *(lb.)*	54.0	55.4
Av. weight gain, birth through 60 days *(lb.)*	45.8 ± 7.0[a]	50.0 ± 9.8
Av. intake of milks *(lb.)*	374.2	388.6
Av. intake of concentrate *(lb.)*	29.7	35.9
Av. intake of hay *(lb.)*	17.9	18.0
	Post-aureomycin feeding period, 61 through 90 days	
No. of calves	6	6
Av. weight, 60 days *(lb.)*	99.9	107.2
Av. weight gain, 61 through 90 days *(lb.)*	36.8 ± 5.2	20.5 ± 14.3
Av. intake of concentrate *(lb.)*	73.2	70.3
Av. intake of hay *(lb.)*	49.0	32.0

[a] Mean ± standard deviations.

Since milks were fed according to body weight, consumption by the experimental group was greater. Concentrate intake averaged 35.9 lb. for the experimental animals, as compared with 29.7 lb. for the controls, but this difference was not significant. Hay consumption was approximately the same for each group.

The average gain of 20.5 lb. by six calves in the experimental group during the 30-day post–aureomycin feeding period was significantly lower ($P < 0.05$) than the average of 36.8 lb. made by the controls. Although concentrate consumption during this period was similar for the two groups, the average daily hay intake of 32.0 lb. by the experimental group was significantly lower ($P < 0.05$) than the intake of 49.0 lb. by animals of the control group.

After aureomycin was removed from the diet at 61 days, two animals in the experimental group continued to gain at above-normal rates, increased concentrate and hay consumption in amounts comparable to that of the control group, and appeared healthy in all respects. The other four individuals of the experimental group gained at subnormal rates ranging from 6.4 to 22.0 lb. for this 30-day period. During the 61- through 90-day interval the average concentrate intake for these four calves remained about constant as compared with that for their 56- through 60-day period; whereas, average hay consumption was lower from 61 through 85 days, but slightly higher from 86 through 90 days. These four calves had diarrhea ranging from mild to severe, the coats became rough, eyes watered, and they lacked general thriftiness during this period.

In Experiment II, seven animals that had received aureomycin through 60 days of age were continued from 61 through 120 days on hay, plus a concentrate mixture containing 50 p.p.m. of crystalline aureomycin hydrochloride. During this period their average gain of 76.1 lb. was not significantly greater than the average 66.4 lb. increase by their pair-mates (Table 2). Although the aureo-

TABLE 2

Summary of average initial body weights, gains, and feed intakes of calves in control and experimental groups, during the 61- through 120-day aureomycin supplementation period and the 121- through 150-day post–aureomycin feeding period

	Control group	Experimental group
	Aureomycin feeding period, 61 through 120 days	
No. of calves	7	7
Av. weight, 60 days *(lb.)*	101.7	105.3
Av. weight gain, 61 through 120 days *(lb.)*	66.4 ± 12.0[a]	76.1 ± 10.8
Av. intake of concentrate *(lb.)*	147.8	166.0
Av intake of hay *(lb.)*	137.3	150.3
	Post–aureomycin feeding period, 121 through 150 days	
No. of calves	6	6
Av. weight, 120 days *(lb.)*	163.1	180.1
Av. weight gain, 121 through 150 days *(lb.)*	33.3 ± 7.7	25.5 ± 13.9
Av. intake of concentrate *(lb.)*	100.3	99.0
Av. intake of hay *(lb.)*	96.7	88.7

[a] Mean ± standard deviations.

mycin-fed animals consumed an average of 12.3% more concentrate and 9.5% more hay than did the controls, these differences were not significant. Gains of the control group averaged 98% of the normal growth rate *(34)*, and gains in the experimental group were 112% of the normal.

Since Pounden and Hibbs *(31)* reported that rumen inoculations of young calves with fresh cud from cows had reduced digestive disturbances and improved health, this was tried with three animals during the 121- through 150-day post–aureomycin feeding period. One calf given cud daily from 121 through 130 days plateaued in body weight during the period of 126 through 143 days of age, but gained the last seven days. Hay intake declined on the 124th day, and concentrate consumption declined on the 130th day. The coat became rough and the animal lost its thrifty appearance. The other two animals fed cud on 19 days during the period of 121 through 140 days appeared healthy and made gains of 33.4 and 23.2 lb., respectively. The smaller gain by the latter calf was due to lower feed intake, resulting from a tongue injury incurred during cud-feeding.

One of the three animals not fed cud during the post–aureomycin feeding period gained 44.3 lb. and appeared healthy. Another gained 27.9 lb. and, although there was a small reduction in feed intake and growth rate from the 125th through the 142nd day, the animal appeared to be in satisfactory health. The third gained only 8.7 lb. Feed intake declined at 128 days and body weight plateaued at 131 days. The coat became rough, the skin thickened and became scaly, eyes were watery, and diarrhea developed.

The average gain of 25.5 lb. made by the six experimental animals receiving these diverse treatments during the 121- through 150-day period did not prove to be significantly lower than that of 33.3 lb. made by the control group.

During the post–aureomycin feeding period, animals that had previously received the antibiotic varied widely in their response, ranging from cases in which gain rate, feed intake, and appearance were above average to some in which there were marked anorexia, some loss of weight, and development of pathological symptoms. Some animals with milder symptoms recovered within a period of a few days to three or four weeks; however, in severe cases recovery was not complete at 50 or 90 days after termination of aureomycin feeding.

These symptoms appear similar to some of the undesirable side effects reported in humans following some treatments with aureomycin. Harris *(12)* and Woods, Manning, and Patterson *(40)* noted that nausea, vomiting, and diarrhea occurred in some patients following treatment with aureomycin and *(12)* that this occurrence was three times more prevalent among females than males. Patients suffered lesions of mucous membranes and, in one case, lesions typical of fungous infection appeared on contiguous areas of the thighs. Weiss is reported *(1)* to have observed similar symptoms and that there had been cases in which a child's entire body was covered with itchy inflammation. They *(1, 12, 40)* indicated that following destruction of intestinal bacterial flora by the action of aureomycin, *Monilia (Candida) albicans*, a normal inhabitant of the bowel, may overgrow, perhaps gather virulence, and then invade tissues.

SUMMARY

Calves fed hay, concentrate, and milks containing aureomycin hydrochloride in amounts equivalent to 45 mg. daily per 100 lb. body weight, from birth through 60 days of age, made average body weight gains of 50.0 lb., as compared with 45.8 lb. by control animals consuming the unsupplemented ration. Calves, previously fed aureomycin, that were continued from 61 through 120 days on a ration of alfalfa hay plus concentrate containing 50 p.p.m. of aureomycin hydrochloride, made average gains of 76.1 lb., as compared with 66.4 lb. by the control group. The differences in average body weight gains and feed intakes between the supplemented and the unsupplemented groups were not significant.

Following removal of aureomycin from the diet of six calves at 61 days of age, the average body weight gain and hay consumption were significantly lower during the ensuing 30 days than for the six animals previously fed the unsupplemented ration. The effects of its removal from the diet at 121 days of age were variable.

After aureomycin was removed from the diet, the responses of individual animals varied widely, ranging from cases in which gain rate, feed intake, and appearance were excellent, to some cases in which there were anorexia, reduced growth, excessive lachrymation, and development of diarrhea, rough coat, and thickened, scaly hide. Some animals with mild symptoms recovered within a period of a few days to a few weeks, and some severe cases had not recovered after 50 or 90 days.

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INFLUENCE OF HEATING ON ARTIFICIALLY INDUCED ANTIBACTERIAL AGGLUTININS IN MILK[1,2]

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The heat inactivation of induced antibodies contained in milk was studied. A technique is described for preparing an acid whey for use in agglutination tests. It was found that inactivation of antibodies specific for Salmonella pullorum was a function of temperature and logarithm of time between 155° and 165° F. The results indicated that pasteurizing and drying milk containing such antibodies does not impair their agglutinating ability. *Editor.*

It has been suggested that an artificial passive immunity might be conferred upon a host by feeding special milk which contained large quantities of specific antibodies. Petersen and Campbell *(6)* have reported success in inducing the production of specific antibodies to a variety of microbial antigens, by an udder infusion technique. The milk from these infused glands was fed to laboratory animals and human volunteers, and in several instances a measurable rise in the antibody titer of the host blood serum was obtained.

Before a prophylactic practice of this nature could become practical, however, precautions would need to be taken to insure the safety of the antibody-containing milk. In certain instances, a live bacterial or viral agent may be used as the stimulating antigen, and the possibility would exist that this pathogenic material could gain entrance to the host via the raw milk. Consequently, to be acceptable, the milk should retain unaltered antibody after being heated sufficiently to destroy pathogens. Furthermore, the antibody material would have to be sufficiently resistant to withstand heating during condensing and drying, if this milk were concentrated and dehydrated.

Few studies have been made on the thermal resistance of antibodies which might be present in milk. Rosenau and McCoy *(8)* have shown that agglutinins in raw milk are destroyed by heating to 80° C., and Chambers *(1)* has verified their work. Hobbs *(5)* described a factor which clumped several strains of *B. coli* in raw and pasteurized milk, but was destroyed at 70° C.

According to the most recent classification of milk proteins, antibodies are catalogued as immune globulins of the serum protein fraction, and might be expected to show thermal denaturation characteristics of this group. Harland, Coulter, and Jenness *(4)* found that heat denaturation of serum proteins was a function of temperature and logarithm of time of heating between 145 and 180° F. Pasteurization sufficient to make milk safe for consumption had essen-

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tially no adverse effect (less than 5% denaturation) on the serum proteins, and if precautions were taken during manufacture, dried milks with little denatured serum protein could be produced *(2)*.

Since a source of milk containing high titers of specific agglutinins was available, an investigation was undertaken to study the effect of heating on the thermal resistance of these materials and to see if antibody-containing milk was suitable for processing.

EXPERIMENTAL METHODS

Milk used in this study was obtained from a 4-year-old Ayrshire cow infused with a phenol-killed suspension of *Salmonella pullorum* cells, during the dry period before parturition *(7)*. The milk was obtained from this cow on the second day of lactation, frozen and stored at −20° F., and representative samples liquefied at 70° F. when needed.[4] Preliminary work showed that there was no significant change in the agglutinin titer during the frozen storage and melting periods.

To obtain a fat-free sample of milk, for the plate agglutination tests used to ascertain the approximate agglutinin titer of the milk and the specificity of milk-borne agglutinins toward *S. pullorum* antigen, the milk was centrifuged at room temperature (4,400 r.p.m. for 15 min.) and the skimmilk fraction was then tested.

The acid whey used for agglutination tests was prepared from the milk in the following manner: One ml. of whole milk was added to 8.6 ml. of distilled water and tempered to 104° F. The casein was precipitated by addition of 0.2 ml. of 10% acetic acid, after which the tubes were allowed to stand at 104° F. for 15 min. The pH was adjusted to 4.6 by the addition of 0.2 ml. of 1 *N* sodium acetate, after which the samples were cooled to room temperature and filtered through S & S No. 589 filter paper. This technique always yielded a clear acid whey (1 : 10 dilution of the original milk), even when early colostrum or mastitic milk was used.

The plate agglutination test was performed by mixing 0.1 ml. of the skimmilk or whey with 0.1 ml. of the appropriate antigen suspension, on a clean glass plate. Agglutination was observed through a 6 × binocular dissecting microscope after 15 min. of incubation at room temperature. When necessary, tenfold dilutions of the original skimmilk fractions were made with distilled water.

In the tube agglutination tests, the whey was diluted serially in distilled water. To 2-ml. quantities of the appropriate whey dilutions, 0.05 ml. of the washed *S. pullorum* suspension was added, and the mixture was then shaken and incubated at 98° F. for 18 hr. before observation.

Freshly washed suspensions of stock laboratory strains of *Escherichia coli*, *Streptococcus lactis*, and *Lactobacillus acidophilus* were used to establish the specificity or nonspecificity of agglutination. The *S. pullorum* antigen used in both plate and tube agglutination trials was a formalin-killed suspension of

[4] Milk was obtained through the courtesy of Dr. W. E. Petersen, Department of Dairy Husbandry.

variant strain 296 [5] Immediately before any trial, the *S. pullorum* antigen was centrifuged and washed six times with sterile saline, and was finally suspended in saline to a density approximating 2 on the McFarland Nephelometer. This precaution was necessary, since preliminary trials indicated that formalin and phenol contributed to nonspecific agglutinations when colostrum or mastitic milk was used.

Heating trials were conducted by dispensing 2-ml. quantities of milk into small, soft-glass tubes, sealing the tubes and immersing them in a water bath held at the desired temperature by thermostatic control. At 5-min. intervals, triplicate tubes were removed from the holding bath and were cooled to room temperature by immersion in cold water, before proceeding with the agglutination tests.

RESULTS

Early in the course of this study, it was recognized that the highest apparent agglutinin titers, according to the plate test, were associated with milks showing abnormal properties such as stringiness and heat-coagulability. These properties were probably the result of the very early stage of lactation and the inflammatory response to udder infusion. The characteristics of the milk interfered with its assay by a technique involving semimacroscopic clumping of bacterial cells. Furthermore, at times this milk contained low but detectable concentrations of nonspecific agglutinating material, which clumped antigens apparently unrelated to the antigen used in infusing the udder. Thus, it was necessary to develop a method which would overcome these difficulties. Numerous attempts were made to coagulate the casein with rennet, acid, or salt, but in very few instances was it possible to obtain a clear whey which retained the agglutinating properties. The final technique of whey preparation, involving acid precipitation of casein, as described under Experimental Methods, was successful.

Applicability of the above method to the problem under study is illustrated (Table 1). In the first place, both fresh and frozen whey retain the approximate titer of the original raw milk. Secondly, the questionable agglutination of the nonspecific antigens was eliminated by this treatment. This table shows that a source of milk containing a high potency of specific agglutinins was available, and that a satisfactory technique for assaying these agglutinins was at hand. Furthermore, since the whey was clear, it could be used in a tube agglutination test which is more precise than the plate method.

Results of the heating trials are shown (Tables 2, 3, and 4). Heating at 155° F. for periods of less than 25 min. had no apparent effect on the titer of agglutinins, as measured by the tube agglutination technique. However, it should be recognized that agglutinin inactivation is probably a gradual function of heat treatment, whereas the assay technique is discontinuous. Thus, a reading of 1:1,600 positive—1:3,200 negative implies that the actual concentration of agglutinins lies somewhere within this range. The true value might be as low as

[5] *S. pullorum* antigen was obtained through the courtesy of Dr. R. Lindorfer, Department of Veterinary Medicine.

TABLE 1

Antigen specificity, as shown by plate agglutination tests on whey and milk from a cow infused with S. pullorum

Sample	Dilutions of milk and whey, showing positive or negative agglutination against antigens indicated								
	S. pullorum			*S. lactis*		*E. coli*		*L. acidophilus*	
	1:10	1:100	1:1,000	1:10	1:100	1:10	1:100	1:10	1:100
Raw whole milk	+	+	+	±	−	±	−	−	−
Raw defatted milk	+	+	+	−		−		−	
Whole milk (boiled 5 min.)	−								
Whey from raw whole milk	+	+	+	−		−		−	
Whey (stored frozen 1 wk.)	+	+	+						

TABLE 2

*Dilutions at which anti–*S. pullorum *agglutinins could be detected by a tube agglutination test, after heating to 155° F. for different time periods*

Time held at 155° F.	1:400	1:800	1:1600	1:3200
(min.)				
Raw	+	+	+	−
5	+	+	+	−
10	+	+	+	−
15	+	+	+	−
20	+	+	+	−
25	+	+	±	−
30	+	+	−	−
35	+	+	−	−
40	+	+	−	−
45	+	+	−	−
50	+	−	−	−
55	+	−	−	−
60	+	−	−	−

1:1,600, or as high as 1:3,199. Unless a series of dilutions within this range were assayed, the true value could only be approximated. Nonetheless, these tables adequately illustrate the exposures at which sufficient agglutinin was inactivated to pass from one range of dilutions to the next.

It can be seen that the inactivation of agglutinins by heat takes place in an orderly fashion. At 155° F., 25 min. of holding were necessary to diminish the original titer to the next serial dilution, and another 20 min. at the same temperature were required to reduce this titer by 50%. At 160 and 165° F., the same general pattern was observed, but the rate of inactivation was proportionally higher.

These results are portrayed graphically (Figure 1), showing that the velocity of inactivation is at its greatest in the initial stages. Subsequent holding periods, however, did not inactivate agglutinins at the same rate, and the rate of inactivation decreased as the titer approached zero. In this regard, these data are similar to the heat-denaturation studies of Harland and Ashworth *(3)* on whey

proteins. These authors showed that the velocity of whey protein coagulation was highest during the first 5 min. of heating, and that this rate gradually diminished during subsequent holding periods, yielding curves which approached, but never reached, 100% denaturation.

In order to define these data in a uniform manner, they were superimposed onto a temperature-log of time graph (Figure 2). This graph shows the original heat exposures required by Harland *et al. (4)* to denature different percentages of serum proteins, and the time-temperature combinations required to inactivate agglutinins. The plot of 50% agglutinin inactivation was derived from the time-temperature combinations needed to reduce the titer to 1:800 (as shown in Figure 1). Similarly, the 75% agglutinin inactivation line was derived from the

TABLE 3

*Dilutions at which anti–*S. pullorum *agglutinins could be detected by a tube agglutination test, after heating to 160° F. for different time periods*

Time held at 160° F.	1:160	1:320	1:400	1:800	1:1,600	1:3,200
(min.)						
Raw	+	+	+	+	+	−
5	+	+	+	+	±	−
10	+	+	+	+	−	−
15	+	+	+	±	−	−
20	+	+	+	−	−	−
25	+	+	+	−	−	−
30	+	+	±	−	−	−
35	+	+	−	−	−	−
40	+	±	−	−	−	−
45	+	−	−	−	−	−
50	+	−	−	−	−	−
55	+	−	−	−	−	−
60	+	−	−	−	−	−

TABLE 4

*Dilutions at which anti–*S. pullorum *agglutinins could be detected by a tube agglutination test, after heating to 165° F. for different time periods*

Time held at 165° F.	1:20	1:40	1:80	1:160	1:320	1:400	1:800	1:1,600	1:3,200
(min.)									
Raw	+	+	+	+	+	+	+	+	−
5	+	+	+	+	+	+	±	−	−
10	+	+	+	+	+	+	−	−	−
15	+	+	+	+	+	±	−	−	−
20	+	+	+	+	±	−	−	−	−
25	+	+	+	±	−	−	−	−	−
30	+	+	+	−	−	−	−	−	−
35	+	+	±	−	−	−	−	−	−
40	+	+	−	−	−	−	−	−	−
45	+	±	−	−	−	−	−	−	−
50	+	−	−	−	−	−	−	−	−
55	±	−	−	−	−	−	−	−	−
60	−	−	−	−	−	−	−	−	−

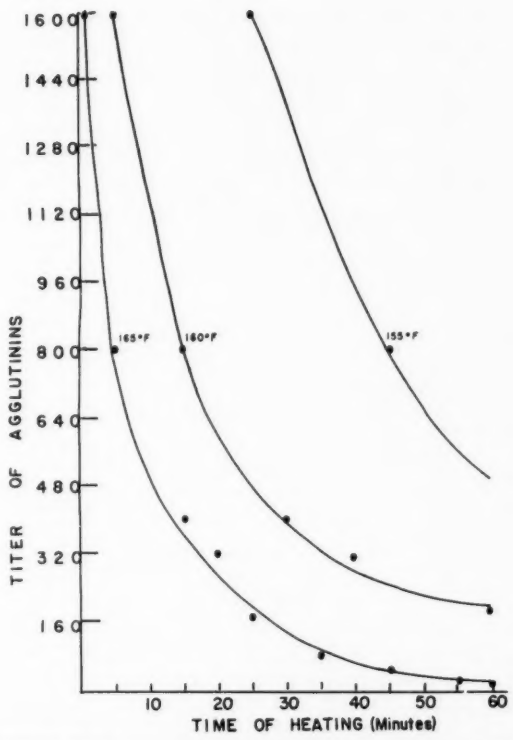

FIG. 1. The influence of different heat treatments on the level of detectable anti-*S. pullorum* agglutinins in milk. (Titer refers to highest dilution of tube agglutination test, showing positive clumping of washed cell suspension.)

exposures (also shown in Figure 1) which yielded a titer of 1:400. It is evident that the agglutinin inactivation curves are related qualitatively and quantitatively to the general serum protein denaturation curves. In other words, it is probable that those thermal factors which influence serum proteins also show the same effect on milk-borne agglutinins. Furthermore, this figure shows that normal pasteurization exposures which are sufficient to render milk safe for human consumption will have no significant effect on the agglutinin titer of milk.

DISCUSSION

Results of this investigation show clearly that agglutinins induced in milk by preparturition infusion of *S. pullorum* respond to heat treatments in the characteristic manner of normal whey proteins. This phenomenon was verified by preparing an acid whey of the heated milks and assaying the agglutinin potency by a tube agglutination test. The specificity of these agglutinins, their thermal sensitivity, and the fact that their presence was induced by mammary infusion of homologous antigen, lend credence to the assumption that these are classical antibodies.

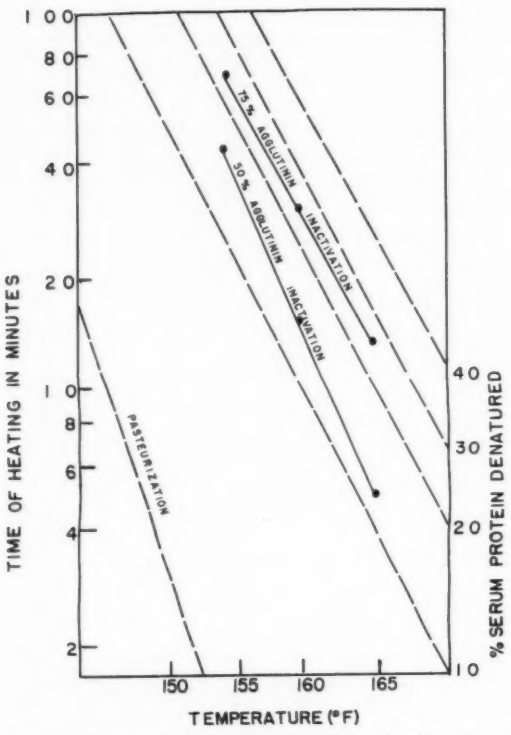

FIG. 2. Thermal exposures required to inactivate 50 and 75% of the anti-*S. pullorum* agglutinins in milk, and the relationship of these values to pasteurization and serum protein denaturation exposures.

The technique employed in preparing a clear whey for antibody assay was readily adaptable for routine studies, and consistently gave satisfactory results. There might be some objection to the fact that the "original" whey was actually a 1:10 dilution of the original milk, and that lower titers of antibody were impossible to measure by this technique. This disadvantage, however, was compensated for by the consistent yield of a clear, stable solution, which had obvious advantages over the cloudy or opaque suspensions resulting from rennet or acid coagulation of the undiluted milk. Furthermore, it might be argued that an antibody titer of less than 1:10 is of no great significance in milk.

It should also be pointed out that the titers obtained on whey, as reported in this paper, are probably lower values than would be obtained in an assay of the heated milk itself, because of the acid precipitation technique employed. Serum proteins are rendered acid-coagulable by heat treatment. Thus, it is conceivable that some antibodies might retain their agglutinating powers after heating, and be available in the milk, but still be sufficiently denatured to be coagulated by acid and removed from the whey during filtration. These anti-

bodies would not be detected by the procedure employed, but could still contribute to the immunizing power of the milk. This possibility, however, is minimized by the observation that antibody inactivation probably proceeded faster on heating than did serum protein denaturation. The latter assumption is derived from the fact that 50% agglutinin inactivation occurred at those exposures required to denature only 10 to 20% of the total serum proteins.

Essentially, the measure of agglutinating antibody can be considered a measure of one specialized group of native serum proteins. Thus, the data presented in this report support the work by Harland, Coulter, and Jenness *(4)*, in which they reported that the denaturation of serum proteins was a function of temperature and logarithm of time. This relationship was expected at the outset of this investigation, but the fidelity of aggreement was surprisingly gratifying.

From a practical point of view, these data indicate the feasibility of pasteurizing milk containing antibody without impairing its antibody potency. If the product should prove to have therapeutic values, there will probably be an attempt to dry it for storage and shipment. Manufacturers can now produce a low-heat, nonfat dry milk solids containing less than 10 to 20% denatured serum protein. These data indicate that they will also be able to prepare a powder containing essentially unimpaired antibody.

SUMMARY

A technique is described for preparing an acid whey which retains the agglutinating power of milk against bacterial antigens. Using this whey in a tube agglutination test, the heat-resistant characteristics of antibodies specific against *S. pullorum* were determined. It was found that these characteristics were essentially the same as those previously reported for the serum protein fraction of normal milk; namely, that their inactivation is a function of temperature and the logarithm of time within the range 155 to 165° F. The values obtained indicate that this milk can be pasteurized and dried without impairing its agglutinating ability.

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A COMPARISON OF SYSTEMS OF FEEDING MILK-REPLACEMENT FORMULAS TO DAIRY CALVES[1]

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The objectives were to eliminate whole-milk feeding, reduce duration of milk-replacer feeding, and improve the starter ration. It was possible to eliminate whole milk after the colostrum-feeding period, to discontinue milk-replacer feeding after 30 days of age, and to use a modified starter in order to raise young dairy calves successfully. *Editor.*

Various complex mixtures have been used successfully as whole milk substitutes for dairy calves *(2, 3, 6, 7)*. The general procedure used in these trials was to feed whole milk for from two to three weeks, then gradually shift to the milk replacement, which was then fed up to 49 days of age. This gradual change to the replacer has been the normal procedure, and no attempt has been made to shift completely to the milk replacement at three days of age.

The present report gives results of attempts to eliminate whole-milk feeding, reduce the duration of milk-replacer feeding, and to improve the companion starter mixture.

EXPERIMENTAL PROCEDURE

Eight calves (males and females), comprising two each of the Ayrshire, Guernsey, Holstein, and Jersey breeds, were assigned to each of the three experimental groups (Table 1) in a randomized block design, replicated by breeds. All calves remained with their dams for three days postpartum. On the fourth day they were placed in individual pens and maintained there until 60 days of age. The schedule of feeding is shown (Table 1). The milk and milk-replacement formula, which was the same formula (No. 4 Trial) reported by Stein *et al. (6)*, was warmed to approximately 100° F. and fed twice daily via open pail.

All calves received U. S. No. 1 alfalfa hay, starter, and water ad libitum from seven to 60 days of age. Calf starters, the composition of which is shown (Table 2), were limited to a maximum of 4 lb. daily. Calves in Groups I and II were fed Starter 1, whereas calves in Group III received Starter 2.

The starters were mixed and pelleted by a commercial mill. The hay and starters were sampled at weekly intervals, and these samples were composited at monthly intervals for chemical analysis, the composition of which is shown (Table 3).

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[2] Farm Crop Insurance Corporation, Raleigh, N. C.

TABLE 1

Experimental groups and schedule for feeding milk-replacement formula (per feeding)

Age	Group I[a]						Groups II and III[b]		
	Milk		Milk Replacer		Water				
	A-H	G-J[c]	A-H	G-J	A-H	G-J	Milk	Milk replacer[b]	Water
(days)	*(lb.)*		*(oz.)*		*(lb.)*		*(lb.)*	*(oz.)*	*(lb.)*
1-3	All calves with respective dams								
4-5	4	3	0	0	0	0	0	8	4
6-10	2	2	6	4	2	1	0	8	4
11-18	0	0	8	8	4	4	0	8	4
19-28	0	0	10	8	5	4	0	8	4
29-30	0	0	12	10	6	5	0	8	4
31-36	0	0	12	10	6	5	0	0	0
37-49	0	0	14	12	7	6	0	0	0

[a] Groups I and II were fed Starter 1 (Table 2) and Group III received Starter 2 (Table 2).
[b] Dry weight basis.
[c] A = Ayrshire; H = Holstein; G = Guernsey, and J = Jersey.

Feed consumption was recorded daily. The calves were weighed at the beginning of the experimental period, at weekly intervals, and at 60 days of age. The calves were examined twice daily for symptoms of diarrhea and other abnormalities. Liquid feeding was reduced by one-half when a calf scoured, and gradually increased to the original level of feeding as the diarrhea subsided. Cases of scours with accompanying body temperatures above 103° F. were treated by intramuscular injections of 500,000 to 1,000,000 units of penicillin.

RESULTS AND DISCUSSION

Growth. Average body weight gains are presented (Table 4). The calves in Group I gained 16% more than calves in Groups II and III combined. Also,

TABLE 2

Composition of the calf starters

Ingredients	Starter 1 (Groups I and II)	Starter 2 (Group III)
	(%)	*(%)*
Ground yellow corn	20.8	20.5
Wheat bran	15.0	15.0
Crimped oats	20.0	17.5
Linseed oil	5.0	5.0
Soybean oil meal (44% solvent process)	15.0	—
Cane molasses	5.0	5.0
Nonfat dried milk solids	5.0	—
Distillers dried corn solubles	5.0	—
Fish meal	—	2.5
Milk-replacer base (Borden's MRB)[a]	—	17.5
Cavpro "200"[b]	—	7.5
Alfalfa leaf meal	7.5	7.5
Dicalcium phosphate	0.5	1.0
Ground limestone	0.5	0.5
Salt	0.5	—
Salt (iodized)	—	0.5
Vitamin A and D_2 meal[c]	0.2	—

[a] A soybean flour-whey soluble product.
[b] A vitamin-antibiotic supplement provided by the Borden Company.
[c] Contains 1,814,544 and 226,800 U.S.P. units of vitamin A and D_2, per lb., respectively.

TABLE 3
Proximate analysis of the experimental feeds (as-fed basis)

Feed	No. samples	Dry matter	Crude protein	Ether extract	Crude fiber	N-free extract	Ash
Milk replacer	2	92.8	31.1	5.1	3.0	44.2	8.9
Starter 1	6	90.4	19.7	4.2	7.1	53.2	6.3
Starter 2	6	90.0	21.5	4.5	6.9	50.2	7.0
Alfalfa hay	4	92.0	17.0	3.0	26.2	38.1	7.8

calves in Group III had an average gain 22% greater than those in Group II. However, one Jersey calf in Group II gained only 0.16 lb. daily. Statistical analysis *(5)* showed no significant differences in mean body weight changes due to diet. The coefficient of variation was 20.08% among calves. The large variation among calves partially explains the lack of statistical significance.

That calves in Groups II and III continued at a uniform rate of gain, during the interim immediately following the cessation of milk-replacer feeding at 30 days of age, is revealed (Table 4). Apparently, the calves were consuming sufficient quantities of starter and hay to continue to grow during this change-over period.

Feed consumption. The simplified system of milk-replacer feeding employed in Groups II and III yielded a net saving of 32.2 lb. of liquid whole milk and 27.5 lb. of milk replacer (dry basis) per calf. Average hay consumption was approximately the same for all groups, but mean starter consumption was 17.1 and 30.8% greater for Groups II and III, respectively, than for Group I. The greater starter consumption by Group III calves apparently was due to the stimulated appetite and growth rate caused by the inclusion of Borden's "Cavpro 200" which contains, in addition to vitamins, an antibiotic supplement which has been demonstrated to give a growth stimulation to young calves when incorporated into the diet *(1, 4)*.

Health. The physical appearance of the calves was similar for all groups. All calves presented a rough coat and a thin appearance during the first four or five weeks of the trial, after which they improved considerably in appearance. Scouring was prevalent in the early part of the trial, but was easily handled by reducing liquid intake. Only four cases of diarrhea, which were accompanied by a fever, required penicillin treatment. With the schedule of feeding of the milk replacer employed in Groups II and III, it appeared that the calves of the smaller breeds were fed excess amounts of the replacer. Perhaps by feeding the

TABLE 4
Average body weight gains of calves raised from four to 60 days of age on milk-replacer systems

Group	No. calves	Days on experiment									Daily gain
		7	14	21	28	35	42	49	56	60	
I	8	1.5	5.4	13.2	21.4	30.4	42.8	54.9	62.8	68.0	1.19
II	8	0.2	2.6	9.9	15.4	23.9	29.9	40.6	48.2	52.8	0.93
III	8	1.1	3.0	11.0	17.8	24.8	35.1	44.4	56.4	64.5	1.13

individual calves more, according to their size and ability to take the milk, the diarrhea would have been reduced.

Under the conditions of this trial, it appears that milk-replacer feeding systems of young dairy calves can be simplified. Whole-milk feeding was eliminated successfully after the colostrum-feeding period, and milk-replacer feeding was discontinued at 30 days of age. Moreover, the system was further improved by modifying the companion starter by replacing soybean oil meal, nonfat dried milk solids, and distillers corn solubles with a special processed soybean flour-whey soluble product and a vitamin-antibiotic supplement.

SUMMARY

Twenty-four calves from four dairy breeds were used in a comparison of milk-replacer feeding systems. Group I received 32.2 lb. whole milk from four to ten days, and 53.2 lb. of milk replacer (dry basis) to 49 days of age. Groups II and III were fed no whole milk after the colostrum-feeding period and an average of 25.6 lb. of milk replacer (dry basis) to 30 days of age. The milk replacer was reconstituted in 100° F. water and fed via open pail. The starter fed to Group III was improved by using a special processed soybean flour-whey soluble product in the place of soybean meal, dried skimmilk, and distillers corn solubles.

Average body weight changes were slightly greater for Group I and lowest for Group II. However, differences were not statistically significant. Alfalfa hay (fed from seven to 60 days of age) consumption was similar for all groups. Starter intake was 80.3, 92.2, and 105.0 lb. for Groups I, II, and III, respectively.

It was possible to eliminate whole milk after the colostrum-feeding period, to discontinue milk-replacer feeding after 30 days of age, and to use modified starter in order to raise young dairy calves successfully.

ACKNOWLEDGMENTS

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EFFECT OF PROCESSING AND STORAGE ON ELECTROPHORETIC PATTERNS OF SKIMMILK PROTEINS

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Electrophoretic analyses revealed protein complexes in milk subjected to different processing. These complexes varied with the type of treatment. Interactions occurred between alpha- and beta-casein; in addition, there was complexing between beta-lactoglobulin and complexed alpha-casein. Milk stored at different temperatures revealed continuous changes. The alpha-casein complex was the most labile and dissociated into two major groups, which changed in concentration during storage. Electrophoretic analyses did not reveal the causes of gelation. *Editor.*

As an approach to the problem of the effect of heat on the storage quality of different forms of milk, electrophoretic studies were carried out on the proteins from skimmilk which had received various commercial treatments. These included preheating, concentration of the milk (3:1), high-temperature, short-time and long-time sterilization, and storage at different temperatures. Various electrophoretic studies have been made on the isolated purified proteins of skimmilk *(1-4, 8-11, 14, 15, 19, 22, 23)*, and other studies have been carried out on the unseparated skimmilk proteins *(13, 16, 17, 21)*. Tobias *et al. (21)* studied skimmilk which had been heated several seconds at 300° F., and Slatter and Van Winkle *(17)* analyzed skimmilks which had received longer heat treatments at lower temperatures. Both noted changes in the protein after heat treatment, the more extreme changes being observed by Slatter and Van Winkle.

EXPERIMENTAL PROCEDURE

Pooled raw milk was skimmed by the Department of Dairy Technology, then given various heat treatments. The milk was first preheated in a tubular heating unit, by bringing it to 242° F. in six seconds. It was held at this temperature for two minutes, then cooled to about 70° F. in six seconds. This was followed immediately by a concentration (3:1) of the milk by vacuum distillation at from 120 to 125° F. The batch was then cooled and held overnight at 40° F. The next day, one portion of the milk was given a high-temperature, short-time sterilization (HTST). The milk was warmed to 130° F. in about ten minutes, then passed through a tubular heating unit which brought the milk to 283° F. in six seconds. The milk was held at this temperature for ten seconds, followed by cooling to about 70° F. in six seconds. The milk was then aseptically canned and stored at 40, 70, and 100° F.

Another portion of the milk received a long-time sterilization (LS) treatment. The milk was canned, placed in an autoclave, and brought to 242° F. in

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20 min. The milk was held at this temperature for 16 min., followed by a 15-min. cooling time to about 70° F. The temperature in the autoclave was determined by use of a maximum-reading thermometer. This milk was stored at 100° F.

Samples of the milk were taken after the various treatments and were diluted with buffer to a protein concentration of about 1%. In addition, samples of the HTST milk, stored at 70 and 100° F., were analyzed each week, until difficulty was encountered in reconstituting the milk. Veronal buffer (0.02 *M* sodium veronal, 0.08 *M* sodium chloride, pH 7.78, 0.1 μ) was used throughout the series. The samples were dialyzed in an ice bath for from three to four days against three changes of buffer. Tobias *et al. (20)* showed that raw skimmilk samples, dialyzed in an ice bath, were electrophoretically unchanged after three days, whereas samples heated at 300° F. for several seconds were unchanged after 14 days.

A slight amount of precipitate was evident in the raw skimmilk sample after dialysis. The solution, after being filtered through No. 5 Whatman paper, was clear. The remainder of the samples showed no obvious precipitates, but were rather turbid and remained so after filtration.

Electrophoretic analyses were carried out at 1° C. in a Tiselius type electrophoresis apparatus manufactured by Frank Pearson Associates. A 10-cc. electrophoresis cell was used, 86 mm. in length, 3 mm. in width, and 25 mm. in depth. A mercury arc (AH4-100W) was used as a light source, with a Wratten 25A filter. Patterns were recorded by the Longsworth scanning technique *(12)*, using Kodak 103 F spectroscopic plates. Areas of the components were determined by use of a planimeter. Due to the complexity of the patterns, rather arbitrary separation of the components was necessary at times. Mobilities were determined from the descending patterns, using the conductivity of the protein solutions at 0° C. Where possible, the components were identified according to the nomenclature suggested by Jenness *et al. (7)*. However, the formation of unknown casein complexes presented a limitation to this procedure.

RESULTS

Electrophoretic analyses of skimmilk samples that received various commercial treatments are given (Table 1 and Figure 1). The sensitivity of the milk system to differences in heat is reflected in these analyses. In the raw skimmilk pattern, five fractions were present, four of which could be fairly well identified. With the preheat treatment and concentration of the milk, the patterns revealed changes in the relative concentrations of the proteins. High-temperature, short-time and long-time sterilization of the concentrated milk produced fractions which were mostly unidentifiable. The leading components were judged to be complexes of alpha-casein on the basis of concentration and mobility. The complexes showed a strong tendency towards dissociation. It was found necessary to label the components alphabetically in order of their mobility, rather than to attempt to identify the fractions from pattern to pattern.

TABLE 1

Effect of processing on skimmilk proteins

Sample	Per cent relative area, ascending		Per cent relative area, descending		Descending mobility 1×10^5 cm^2/sec. volt
Raw skimmilk proteins	[a]A	5.4	A	1.7	−1.6
	B	23.2	B	21.4	−2.8
	C	3.3	C	4.9	−3.9
	D	14.3	D	10.7	−4.7
	E	53.8	E	60.3	−5.9
Preheated (heated 242°F., 2 min.)	A	3.0	A	2.3	−1.4
	B	23.9	B	17.2	−2.9
	C	3.6	C	3.6	−4.0
	D	69.5	D	11.9	−5.2
			E	65.0	−6.1
Preheated, conc. (3:1)	A	6.7	A	2.1	−1.6
	B	14.8	B	11.3	−2.9
	C	3.4	C	3.6	−3.8
	D	75.0	D	83.0	−5.7
Preheated, conc. (3:1); heated 283°F.—10 sec. (HTST)	A	5.3	A	3.2	−1.3
	B	21.9	B	16.8	−2.6
	C	11.8	C	2.6	−3.5
	D	61.0	D	13.0	−4.3
			E	19.2	−5.0
			F	45.2	−5.8
Preheated, conc. (3:1); heated 242°F.—16 min. (LS)	A	3.1	A	2.5	−1.8
	B	11.1	B	10.0	−2.8
	C	6.6	C	1.7	−3.4
	D	34.2	D	7.4	−4.1
	E	44.9	E	39.2	−4.8
			F	39.2	−5.7

[a] Fractions listed alphabetically according to increasing mobility.

The untreated raw skimmilk in the descending pattern contained 60% of a fraction considered an alpha-casein complex (Fraction E) and 21% of beta-casein (Fraction B). Gamma-casein (Fraction A) was present in small amounts and showed better resolution in the ascending than in the descending pattern. Fraction D, comprising 11% of the protein, was considered to be beta-lactoglobulin. From its mobility and concentration, it appeared to be uncomplexed with alpha-casein. Less than 5% of the protein (Fraction C) had a mobility intermediate between beta-casein and beta-lactoglobulin. This could conceivably be whey protein or alpha-casein complexed with slower-moving fractions of casein.

Tobias *et al. (21)* and Slatter and Van Winkle *(17)* analyzed raw skimmilk proteins in veronal, pH 8.6, 0.1 μ, and borate-NaCl, pH 8.4, 0.1 μ, respectively. The very low mobility (-4.7×10^{-5} cm^2/sec volt) obtained for the alpha-casein complex in borate buffer indicated interaction of the buffer with this protein. Tobias *et al.* found a mobility of -6.35×10^{-5} cm^2/sec volt for the alpha-casein fraction, simlar to the value of -5.9×10^{-5} cm^2/sec volt found in the present study using veronal-NaCl, pH 7.8, 0.1μ. The relative concentrations of the protein fractions obtained in veronal buffer by Tobias *et al.* were in the same range as the present study. However, in the veronal-NaCl buffer, an additional minor component was evident.

The raw, unconcentrated skimmilk was preheated at 242° F. for 2 min. Analysis of this sample showed a partial disappearance of the beta-lactoglobulin. It no longer existed as a separate peak in the ascending pattern, but a slight

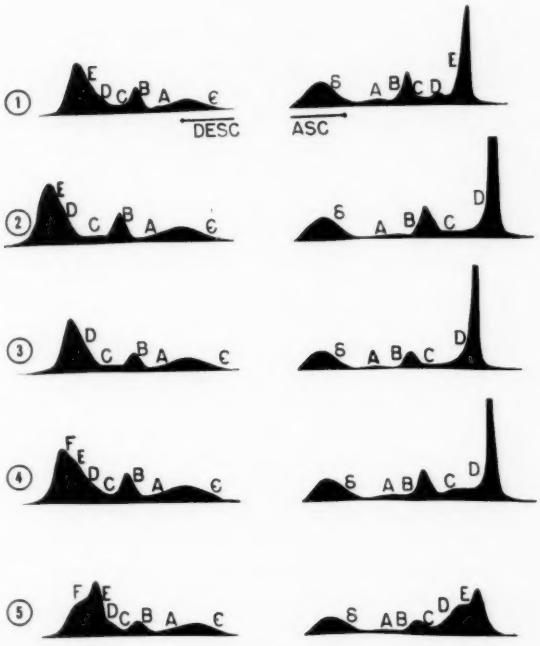

FIG. 1. Electrophoretic patterns of skimmilk after processing.

1. Raw skimmilk protein; 2. preheated (242° F., 2 min.); 3. preheated conc. (3:1); 4. preheated, conc. (3:1), HTST (283° F.—10 sec.); 5. preheated, conc. (3:1), LS (242° F.—16 min.).

Av. E: 5.2 volts/cm; av. T: 12,310 sec.

Buffer: Na veronal–NaCl, pH 7.8, 0.1 μ.

asymmetry (Fraction D) on the alpha-casein complex in the descending pattern could be seen. The absence of the peak in the ascending pattern was reflected in an increase in the concentration of the leading component. The beta-casein (Fraction B) showed a slight decrease in concentration in the descending pattern, but practically none in the ascending. The mobility of the major components remained essentially unchanged. The question arose as to whether the beta-lactoglobulin was actually complexed with the alpha-casein, or whether it was merely travelling with it unresolved.

Sedimentation studies *(18)*, using radioactive techniques, strongly support the interaction of casein with whey proteins on heat treatment. Sedimentation of raw skimmilk removed 7% of whey proteins, whereas sedimentation of heated milk removed 52%. The rate of sedimentation was approximately the same, since when 50% of the casein was removed, 60% of the centrifugable whey proteins was found in the gels. The sedimentation rate of casein micelles would be expected to differ from that of aggregated whey proteins if there were no

interaction with the casein. Further support for casein-whey protein interaction on heat treatment is given by the electrophoretic studies of McGugan *et al. (14)* and Swanson *(19)*. However, the electrophoretic studies were made in purified systems.

Further changes were evident when the milk was concentrated (3:1). The leading component (Fraction D) became increasingly symmetrical and increased in concentration. There was a corresponding decrease in beta-casein (Fraction B), the beta-casein being reduced to half of its original value in the descending pattern. Concentrating the protein appeared to increase the interaction of alpha- and beta-casein. Further evidence of complexing was shown by the reduced mobility of the leading fraction. The mobility of the remaining free beta-casein (Fraction B) was unaffected.

With HTST-concentrated milk, changes were again evident in the protein components. The alpha-casein complex in the descending pattern showed a tendency to dissociate. In addition, there was an increase in the amount of beta-casein (Fraction B), indicating that the heat treatment had weakened the alpha-beta–casein complex formed by concentrating the protein. Despite partial dissociation, the leading fraction of the alpha-casein complex (Fraction F) showed no further change in its descending mobility. However, the components which had dissociated from the leading peak (Fractions D and E), had substantially slower mobilities. While these fractions, from their relative concentrations, undoubtedly contained alpha-casein, the nature of the changes in the protein was unknown. The possibility of partial dephosphorylation of the alpha-casein complex by heat treatment is suggested by the work of Howat and Wright *(5, 6)*, who studied the effect of heat on phosphorus cleavage from casein. Such a dephosphorylation would result in fractions of decreased mobility. Whether such a mechanism is actually related to the observed decrease is problematical.

The sample of milk which had received the LS treatment after the (3:1) concentration, showed a different pattern from that of the HTST sample. Unlike HTST-treated milk, the complexing of alpha-beta–casein was not diminished by this heat treatment. Rather, it was the only pattern in which the concentration of beta-casein was the same in both descending and ascending arms, indicating a stable union with the alpha-casein. The relative concentration of beta-casein (Fraction B) was similar to that of the concentrated unsterilized milk. The two major fractions were present as the leading components and were similar in mobility to the dissociated leading fractions in the HTST-concentrated milk. Whether this represents the same type of reaction, but more extreme in the case of LS-concentrated, is difficult to say.

Analyses of 3:1 concentrated HTST milk stored six weeks at 70° F. are shown (Table 2 and Figure 2). In the sample stored two weeks at 70° F., the general shape of the descending pattern was similar to the unstored HTST sample. Convective disturbances were seen in the ascending pattern. The two weeks of storage resulted in a decrease in beta-casein (Fraction B) and an increase in the leading component (Fraction F). With the third week of

TABLE 2 (Continued)

Effect of storage temperature on concentrated skimmilk protein

Time of storage	Sample	Temp.	Per cent relative area, ascending		Per cent relative area, descending		Descending mobility 1×10^5 cm^2/sec. volt
	conc. 3:1		*A	4.1	A	1.3	−1.5
	HTST		B	18.2	B	11.2	−2.6
			C	2.5	C	1.6	−3.3
			D	15.3	D	8.2	−4.0
			E	3.9	E	22.1	−4.7
2 weeks		70° F.	F	56.0	F	55.6	−5.7
			A	3.6	A	1.9	−1.3
			B	17.4	B	15.7	−2.9
			C	3.0	C	9.1	−3.8
			D	18.3	D	2.5	−4.5
			E	57.6	E	22.5	−5.3
2 weeks		100° F.			F	48.3	−6.2
			A	5.7	A	3.0	−1.4
			B	18.9	B	19.0	−2.9
			C	2.7	C	2.4	−3.9
			D	11.8	D	36.1	−5.3
			E	8.9	E	39.5	−6.1
			F	6.7			
3 weeks		70° F.	G	45.8			
			A	7.3	A	1.8	−1.8
			B	16.9	B	15.3	−2.9
			C	4.2	C	2.6	−3.8
			D	1.5	D	35.3	−5.1
			E	9.3	E	44.9	−6.2
3 weeks		100° F.	F	40.7			
			A	6.1	A	2.8	−1.5
			B	19.8	B	14.1	−2.7
			C	2.5	C	2.7	−3.6
			D	9.4	D	7.2	−4.5
			E	12.1	E	37.6	−5.4
			F	9.6	F	35.6	−6.0
			G	8.0			
4 weeks		70° F.	H	32.5			
			A	4.2	A	1.8	−1.3
			B	16.7	B	10.5	−2.7
			C	4.5	C	2.0	−3.5
			D	39.7	D	47.1	−5.0
4 weeks		100° F.	E	34.8	E	38.5	−6.0
			A	7.3	A	3.5	−1.3
			B	21.3	B	13.6	−2.9
			C	2.7	C	4.6	−4.2
			D	7.5	D	36.4	−5.5
			E	30.6	E	41.9	−6.2
5 weeks		70° F.	F	30.6			
			A	3.4	A	2.2	−1.7
			B	15.9	B	8.8	−2.8
			C	2.4	C	2.8	−3.4
			D	37.3	D	34.2	−4.6
5 weeks		100° F.	E	41.0	E	52.3	−5.7
			A	6.8	A	2.4	−1.4
			B	19.2	B	11.8	−2.7
			C	3.8	C	3.8	−3.8
			D	22.8	D	5.8	−4.6
			E	6.1	E	32.4	−5.4
6 weeks		70° F.	F	41.3	F	43.8	−6.1
			A	5.8	A	1.9	−1.6
			B	20.3	B	13.5	−2.7
			C	6.8	C	3.0	−3.5
			D	27.0	D	7.4	−4.3
			E	39.7	E	29.9	−5.1
6 weeks		100° F.			F	47.2	−6.0

TABLE 2 (Concluded)

Effect of storage temperature on concentrated skimmilk protein

Time of storage	Sample	Temp.	Per cent relative area, ascending		Per cent relative area, descending		Descending mobility 1×10^5 cm^2/sec. volt
			A	6.3	A	2.9	−1.4
			B	21.1	B	13.7	−2.6
			C	5.1	C	2.5	−3.5
			D	14.5	D	8.5	−4.3
			E	8.7	E	34.3	−5.0
			F	10.9	F	38.1	−5.8
6 weeks		40° F.	G	33.4			
	conc. 3:1		A	7.3	A	3.1	−1.5
	LS		B	14.9	B	11.1	−3.0
			C	9.6	C	4.1	−3.9
			D	7.3	D	12.5	−4.8
			E	26.0	E	22.8	−5.3
6 weeks		100° F.	F	34.9	F	46.4	−6.1

[a] Fractions listed alphabetically according to increasing mobility.

storage, there appeared to be a dissociation of this component with an increase in the beta-casein. In the descending pattern, the leading components consisted of two major groups (Fractions D and E) having increased mobilites, while in the ascending pattern, further dissociation was evident.

The fourth week of storage showed continued dissociation of the leading alpha-casein complexes. There was substantially more beta-casein (Fraction B) in the ascending pattern than in the descending pattern. This held true in the fifth and sixth week of storage, as well. The principal change in the fifth week of storage was the formation of two fast-moving fractions of relatively equal concentration in the ascending arm. The dissociation of the leading peak in the descending pattern appeared to have reached a maximum in the fourth week. With continued storage, there was a reversal of this trend, with the fastest fraction increasing in concentration. The final week of storage showed a further shift in the components, with the components between the beta-casein (Fraction B) and the leading alpha-casein complex (Fraction F) showing continued changes.

Descending patterns of the 70 and 100° F. HTST-concentrated stored milk (Figures 2 and 3) showed marked similarity in their general shape. There was dissociation of the alpha-casein complex in both groups, with the dissociation reaching a maximum at the fourth week of storage, then tending to reverse. The 100° F. stored milk showed a consistent decrease in the beta-casein (Fraction B) in the descending pattern until the last week of storage, when an increase was seen. This was not reflected in the ascending pattern, for here the beta-casein concentration remained relatively constant on storage until the last week. The ascending patterns of the 100° F. stored milk differed somewhat from the 70° F., for the alpha-casein complexes of the latter had a greater tendency towards dissociation into several fractions, rather than into two major groups. The mobilities showed some variations, but no consistent differences.

A sample of HTST-concentrated milk stored six weeks at 40° F. was compared with those stored at higher temperatures (Table 2). The pattern of the milk stored at 40° F. was closest to the HTST milk stored four weeks at 70° F.

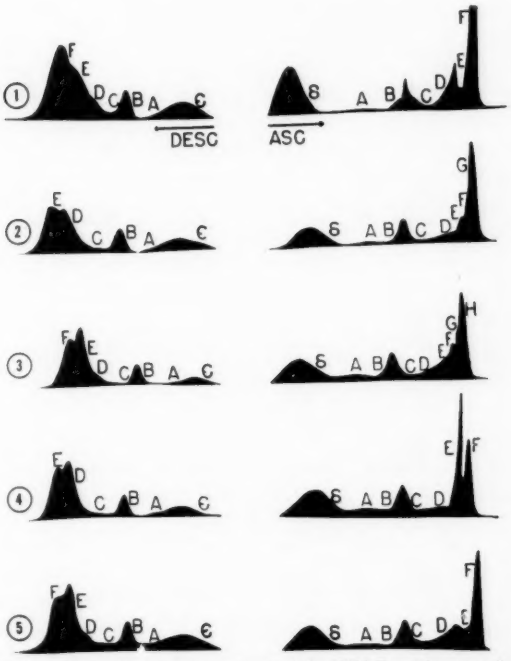

FIG. 2. Electrophoretic patterns of concentrated, HTST milk, stored at 70° F.

1. Second week of storage; 2. third week of storage; 3. fourth week of storage; 4. fifth week of storage; 5. sixth week of storage.

Av. E: 5.2 volts/cm.

Av. T: 13,210 sec.

Buffer: Na veronal—NaCl, pH 7.8, 0.1 μ.

In general, it showed characteristics quite similar to the milk stored at higher temperatures. The leading components in the descending pattern consisted of two major groups, whereas the ascending pattern showed somewhat greater dissociation. An analysis was also made of a sample of LS milk stored at 100° F. for six weeks. An interesting comparison was made between this sample and the HTST milk stored under identical conditions. The mobilities of the components of the HTST milk were very close to those of the LS milk; in addition, the distribution of the components of the two samples was surprisingly similar, considering the substantial differences that existed between the two samples after processing and before storage. The LS milk, when analyzed after heat treatment, showed marked dissociation of the alpha-casein complex. After prolonged storage at 100° F., the leading alpha-casein complex had increased in concentration, whereas the neighboring fraction had decreased.

Despite the similarities in the electrophoretic analyses of the HTST and LS milks stored for six weeks, a wide difference existed in the samples, for the former was rapidly approaching gelation, and the latter was not.

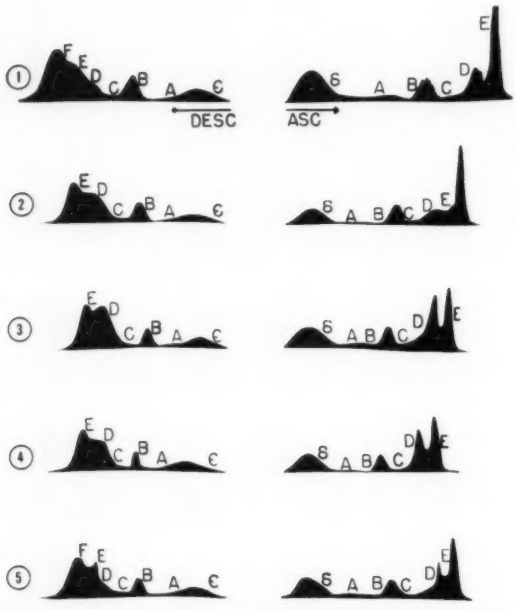

FIG. 3. Electrophoretic patterns of concentrated, HTST milk, stored at 100° F.

1. Second week of storage; 2. third week of storage; 3. fourth week of storage; 4. fifth week of storage; 5. sixth week of storage.

Av. E: 5.1 volts/cm.

Av. T: 12,810 sec.

Buffer: Na veronal—NaCl, pH 7.8, 0.1 μ.

DISCUSSION

The variety of complexes which the milk proteins are capable of forming was demonstrated by electrophoretic analyses of milk that had received different processing treatments. It had been known *(8, 9)* that alpha- and beta-casein complex in solutions of purified whole casein. In the dialyzed samples of the milk which had received concentration and sterilization treatments, there were variations in the relative concentrations of beta-casein (Fraction B). This would indicate that the processing procedures used in this study produced changes that were not completely reversible. In addition to the alpha-beta casein interaction, there also appeared to be an involvement of beta-lactoglobulin with the alpha-casein complex, when the milk was heated sufficiently. The samples that had received heat treatment showed a disappearance of the beta-lactoglobulin peak (Fraction D) that was present in the unheated sample that contained the raw skimmilk proteins. Sedimentation studies *(18)* on skimmilk, and electrophoretic studies with purified systems of alpha-casein and beta-lactoglobulin *(14, 19)*, tend to support the theory of this interaction.

Protein-complexing was evident in the heated, concentrated skimmilk, varying with the type of heat treatment. The HTST treatment appeared to weaken the complex of alpha- and beta-casein, resulting in an increase of free beta-casein (Fraction B). The LS treatment, however, appeared to stabilize the complex, shown by the fact that beta-casein did not increase in concentration in the ascending pattern, as it had with the HTST treatment.

Consideration must be given to the conditions under which the analyses were carried out. Electrophoretic analyses of milk proteins in artificial buffer systems are quite foreign to the normal environment of the proteins. The possible influence of the buffer should not be neglected.

Analyses of the milk stored at different temperatures showed continual changes in the protein fractions. The samples stored at different temperatures, which had received the same heat treatment, showed the same general trends, but differing in degree. Even the LS milk, considered the most stable sample, showed changes after being stored for six weeks. The most labile fraction throughout the storage series was the alpha-casein complex, which in the descending pattern dissociated into two major groups. Whereas the mobility of these two groups did not show substantial change during storage, the concentrations of the two fractions showed constant change. The slower group gradually increased in concentration to a maximum, while the faster group decreased. At about the fifth week, a reversal was noted, with the faster group increasing and the slower group decreasing. A very interesting observation was made between the HTST and LS milks stored at 100° F. for six weeks. The mobility and distribution of the components appeared quite similar and yet the HTST milk was rapidly approaching the point where it could not be reconstituted, whereas the LS milk was still stable. The factors involved in the gelation of milk are quite complex and can not at present be determined from the changes in the milk protein shown by electrophoretic analyses.

SUMMARY

Electrophoretic studies were made on skimmilk proteins after they had received various commercial treatments. Protein interactions were evident and varied with the type of processing.

Concentrated, sterilized milk was stored for six weeks, at temperatures of 40, 70, and 100° F. When samples were electrophoretically analyzed each week, the milk proteins displayed continual changes in the nature and distribution of the components. Similar reactions appeared to be occurring at all three temperatures, but differed in degree.

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MAGNESIUM IN THE CASEIN-CONTAINING COLLOID OF MILK [1]

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The purpose of this work was to determine the magnesium removed from milk by centrifugation, to verify the ratio of calcium removed to magnesium removed, and to establish whether such magnesium is part of the casein complex or is in the form of magnesium citrate. About one-third of the magnesium in milk was accounted for in the casein colloid, none as magnesium citrate, and the calcium-to-magnesium mol ratio in the colloid was about 15:1. *Editor.*

The magnesium content of milk reported in the literature varies from a minimum of 0.0092% to a maximum of 0.0236% *(3, 11)*. There is also considerable variation in figures reported for the amount of magnesium in milk that is insoluble in the colloidal system. Reported nondialyzable magnesium ranges from 13 to 38% of the total *(1, 7)*, and of magnesium retained by the Pasteur-Chamberlain filter tube, from 8 to 34% of the total *(11)*. De Kadt and Van Minnen *(3)* found by centrifugation appreciably less variation in the magnesium content of milk and in the proportion of the total magnesium that is removed as part of the colloid system. Although they suggested that magnesium was thrown out as magnesium citrate, Ford *et al. (5)* considered the centrifugable magnesium as part of the calcium-caseinate–calcium phosphate complex, present in a molar concentration $\frac{1}{15}$th that of calcium.

This work was undertaken to determine more precisely the amount of magnesium removed by centrifugation, to check the calcium : magnesium ratio used by Ford *et al. (5)*, and to establish, if possible, whether the magnesium is present as part of the casein complex or as magnesium citrate.

EXPERIMENTAL PROCEDURE

Sample preparation: Two methods were employed for preparing samples. In the first, the colloids were separated in a centrifuge having a large (50-ml.) air-driven bowl rotor, twice redispersed in water, and redeposited; these washed colloids were then analyzed for magnesium, calcium, and citrate content. This separation and washing technique has been described elsewhere *(5, 8)*. Such washed colloids were first prepared by Ramsdell and Whittier *(9)*, who used a Sharples supercentrifuge for the separation, however.

The second method of preparation was the depletion technique described by Ford *et al. (5)*, using the 50-ml. bowl rotor and analyzing progressively depleted liquid samples for calcium, magnesium, and nitrogen.

Methods of analysis: Magnesium was determined by the method of Hunter *(6)*, citrate by the method of Deysher and Holm *(4)*, soaking the washed colloid in N/30 NaOH, and nitrogen and calcium as described by Ford *et al. (5)*.

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TABLE 1

Results of analysis of various double-washed centrifugally deposited colloids

Type of milk	Calcium	Magnesium	Mol ratio, calcium : magnesium
	(%)	(%)	
Jersey No. 1	2.55	0.131	11.7
Jersey No. 2	2.67	0.120	13.7
Jersey No. 3	2.90	0.120	14.5
Herd No. 1	2.81	0.110	15.3
Herd No. 2	2.69	0.101	16.0
Holstein No. 1	2.86	0.120	14.3
Holstein No. 2	2.72	0.096	17.0
Averages	2.74	0.114	14.6[a]

[a] This corresponds to a weight ratio of 24.0:1.

RESULTS

A summary of the analyses of double-washed complex is given (Table 1). No citrate was detected in any of these double-washed colloids.

Figure 1 shows the relationship between calcium and magnesium content and nitrogen content on liquid samples, as the colloids are progressively removed by centrifugation. It can be seen that both calcium and magnesium are being thrown out along with nitrogen, in approximately constant ratios. The average mol ratio of calcium removed to magnesium removed, given by the slopes of these lines, is 12.4:1. The values plotted are the differences between analyses on the original skimmilk and on the centrifuged serums.

DISCUSSION

The mol ratios obtained for both deposits and serums are of the same order of magnitude as those calculated from the data of De Kadt and Van Minnen *(3)*.

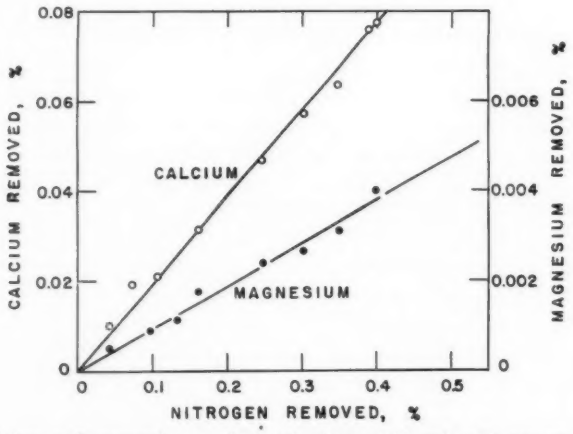

FIG. 1. Calcium and magnesium analysis of ultracentrifuged milk serums. Open circles indicate averages of two or three calcium determinations. Closed circles indicate averages of four or five magnesium determinations.

They reported six analyses of skimmilks and corresponding centrifuged serums, from which mol ratios of 15.0 to 21.5 (average 18.3) can be calculated, and two analyses of unwashed deposits, giving mol ratios of 18.2 and 15.6. Ford, Ramsdell, and Landsman (unpublished data) obtained the ratios 13.8 and 16.9 by analysis of unwashed and washed deposits.

De Kadt and Van Minnen's suggestion, that magnesium is thrown out as magnesium citrate, is neither supported nor completely ruled out by the present results. Their analyses were on unwashed deposits, and since our washed deposits give no test for citrate, but do contain magnesium, it must be concluded that such magnesium is not present as magnesium citrate. However, De Kadt and Van Minnen's unwashed deposits contain more citrate than can be accounted for as citrate proportional to the entrained serum water. More elaborate experiments will be required to determine the condition of this excess citrate.

If milk averages 3% casein-containing colloid and the colloid is 0.114% magnesium, then milk contains 0.0033% magnesium associated with the colloid. Taking the total magnesium content to be 0.012% [based on data given in References *(3)* and *(11)*, and on an average value of 0.011% given by analyses run in this laboratory], then about one-third of the magnesium in milk is in combination with the colloid. This compares with De Kadt and Van Minnen's 25% for the amount of the magnesium that is thrown out by centrifugation.

Reference has been made to data, reported by Van Slyke and Bosworth *(11)*, on the amounts of calcium and magnesium retained by Pasteur-Chamberlain filter tubes. These authors also give results of analysis of the original skimmilks. Therefore, calcium to magnesium ratios for the retained colloids can be calculated from their data. (Columns 2 and 3 of Table 2 are calculated from Table 3 of the article by Van Slyke and Bosworth. Column 4 is Column 2 divided by Column 3, and multiplied by the atomic weight factor, 24.3:40.1.)

Noting that De Kadt and Van Minnen's figures indicate 25% of the magnesium to be colloid-bound, that dialysis experiments *(1, 7)* indicate 13 to 38%, and that our data indicate about 33%, it is suggested that, since Van Slyke and Bosworth's high calcium-to-magnesium ratios always coincide with low retention of magnesium and of calcium by their filters, it is probable that in these cases the pores were too coarse to properly retain the casein-containing colloid. It is suggested, therefore, that these particular ratios can be ignored. The remaining results are much more consistent among themselves; and the indicated calcium: magnesium ratios at high retention values are in good agreement with the results presented here.

In a recent article, Van Kreveld and Van Minnen *(10)* state that one-sixth of the magnesium in milk is present in ionic form. Christianson, Jenness, and Coulter *(2)* report 0.82 to 0.85 millimoles of magnesium ion in a liter of milk. Since there is, on the average, a total of about 5 millimoles of magnesium in a liter of milk, their figures also indicate that about one-sixth of the magnesium is present in the ionic form. Thus, substracting one-sixth (present as ions) plus one-third (associated with the colloid) from the total, there remains about one-half of the magnesium in milk to be accounted for in some other form.

TABLE 2

Calcium : magnesium ratios for the retained colloids [a]

1 Cow No.	2 of total calcium retained	3 of total magnesium retained	4 mol ratio calcium : magnesium in retained colloid
	(%)	(%)	
1	54	8	40.7
2	63	14	27.8
3	60	21	13.0
3	66	8	44.5
4	77	30	12.4
5	62	20	16.4
5	64	9	37.5
6	74	21	19.2
7	64	16	24.7
7	70	28	13.1
8	71	23	18.5
9	71	26	14.4
10	61	16	19.5
11	74	29	12.7
12	75	36	11.1
13	69	31	12.5

[a] Calculated from the data of Van Slyke and Bosworth.

SUMMARY

It has been shown that about one-third of the magnesium in milk is present in the casein-containing colloid, and that the calcium-to-magnesium mol ratio in the colloid is about 15:1.

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REPEATABILITY OF DAIRY COW BUTTERFAT RECORDS IN NEW ZEALAND

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Three components of variance methods have been used to estimate repeatability of butterfat records. The first shows that over all herds, the year effect is negligible; whereas, the year-by-herd interaction is important. On a within-herd basis the estimate of repeatability is higher when allowance is made for year effects than when they are neglected. *Editor.*

In assessing the effect on a selection program of changes in the method of estimating lactation yields, it is important to determine the repeatability of the records based on the different methods. So far, most of the studies of repeatability of butterfat yield have been made on 305-day lactation yields, calculated from milk weighings and samplings made once a month; in almost all cases, the value obtained has been approximately 0.4. The values reported in the present paper were estimated primarily as a basis for examining the relative merits of testing systems based on monthly and on alternate monthly herd tests. The values based on the monthly system are published here, but it is intended to report fully, in a later paper, on the comparison between the two systems of testing.

The value of a cow's record as an indication of her genetic merit is considerably enhanced if it is quoted in conjunction with the level of her herdmates in the same year. By quoting a cow's record in terms of a deviation from the herd average, some account is taken of the environmental conditions common to the whole herd which may have affected her record. These include the year-to-year variation in climatic conditions, which can play a big part in influencing production records, particularly under New Zealand conditions of grassland farming. The increase in repeatability when the variance due to within-herd, year-to-year environmental variation is taken into account, has therefore been studied. Legates and Lush *(3)* and Bonnier *(1)* have also studied this increase. Legates, using lactation records ranging from 270 to 305 days in length, found that repeatability increased from 0.412 to 0.459 when within-herd, year-to-year variations were taken into account. Bonnier, using 445 yearly records of 100 cows, each with 3 to 7 records, found that repeatability increased from 0.224 to 0.299 when yield was treated as a deviation from the herd average.

One of the first estimates of repeatability made from the data of the Herd Improvement Department of the New Zealand Dairy Board was from an analysis of 1,525 mature lactations of 339 cows in 26 pedigree Jersey herds. These records covered some eight years, and no attempt was made to eliminate year effect;

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treating years as replications, repeatability was estimated on a within-herd basis, as 0.40.

A further estimate was one obtained from 1,500 production records analyzed according to age. The ages of the cows in this sample ranged from two to seven years, and the correlation of the production in each age group with the production in every other age group was calculated (two-year with three-year, four-year, and so on). This involved 15 correlation coefficients, the estimates of which are shown (Table 1).

TABLE 1

Correlations between butterfat productions of the same cow at different ages, calculated from a total of 1,500 records

Age in years	Age in years				
	3	4	5	6	7
2	0.67	0.61	0.59	0.53	0.43
3		0.67	0.62	0.58	0.54
4			0.65	0.62	0.57
5				0.67	0.60
6					0.66

In calculating these correlation coefficients, all records of all cows were grouped together, no allowance being made for individual herd effects. The values indicate that the correlation between pairs of records of a cow decreases as the time interval betwen the records increases. The correlation coefficient between two-year-old and three-year-old records is 0.67, and between two-year-old and seven-year-old is only 0.43. Now, repeatability is the intraclass correlation among production records of the same cow. An approximation to this is the average of interclass correlations, age by age, weighted by the number of pairs of records of each combination of ages which are likely to exist in a random sample of dairy cows. Using the figures in this table, such a method gives an estimated repeatability of 0.61.

DATA AND ANALYSIS

The data used for the present study were 5,557 lactation records from 2,436 Jersey cows in 30 herds, with lactations ranging from 100 to 305 days in length. Five herds were chosen at random from each of the six Herd Improvement Associations which are responsible for all herd-testing work in New Zealand. The records, which are based on monthly weighings and samplings, were all made during the four dairying years, 1951/2 to 1954/5. Of the 2,436 cows, 867 had one lactation during the study, 577 had two lactations, 432 had three, and 560 had four.

To take some account of the effect of age on the records, it was necessary to correct the records of the younger cows. This had not been necessary in either of the two earlier analyses, where in one case the data were all mature records and in the other the analysis was age-upon-age regressions. To preserve the constant coefficient of variation, generally recognized as existing among

TABLE 2
Age correction factors

Age	Factor
2 years	1.341
3 years	1.176
4 years	1.068

dairy records, it was decided that age corrections for the present analysis should be multiplicative. Because of the seasonal pattern of dairying in New Zealand, where practically all cows calve in the months of August and September (which constitute New Zealand's spring), it is necessary to consider age to the nearest year only, in applying correction factors. The factors were calculated from the data themselves, on the gross comparison method, by expressing the five- to nine-year-old average as a ratio (greater than 1) of the two-, three-, and four-year-old averages; the factors are shown (Table 2).

A summary of the data for the four years, giving the average milk and butterfat production (mature equivalent), is shown (Table 3).

For all herds together, an over-all analysis was made taking herd effects into account. Then, for each herd separately, two analyses were made; in one, the years were neglected and treated merely as replications; in the other, year effects were estimated for each herd and eliminated from the data, prior to estimating repeatability. For both these within-herd analyses, pooled estimates were also obtained, by combining all herds together to give pooled repeatability figures.

Between-herd analysis. This analysis is based on the model that x_{ijk}, the record of cow j in the i^{th} herd in year k is

$$x_{ijk} = \underset{\text{(Mean)}}{\mu} + \underset{\text{(herd)}}{h_i} + \underset{\text{(cow)}}{c_{ij}} + \underset{\text{(year)}}{s_k} + \underset{\text{(herd by year interaction)}}{(hs)_{ik}} + \underset{\text{(error)}}{e_{ijk}}.$$

Variance components were estimated by Method 1 of Henderson *(2)*, and repeatability of a cow's record in the same herd was then taken as $(\sigma^2_h+\sigma^2_c)/\sigma^2$ where σ^2 is the total variance.

Within-herds, years as replications, analysis. The linear model assumed here is that x_{jk}, the record of cow j in year k is

$$x_{jk} = \underset{\text{(mean)}}{\mu} + \underset{\text{(cow)}}{c_j} + \underset{\text{(year plus error)}}{e_{jk}}.$$

TABLE 3
Summary of data for the four years

Years	No. of cows	Milk (M.E.)	Test	Fat (M.E.)	Day. in mi.k
		(lb.)	*(%)*	*(lb.)*	
1951/52	1,361	6,495	5.27	342	254
1952/53	1,367	6,666	5.31	354	255
1953/54	1,387	6,489	5.26	341	256
1954/55	1,442	6,645	5.27	350	256

This was applied to each herd separately; variance components σ^2_c and σ^2_e were estimated in the customary manner, and repeatability taken as $\sigma^2_c/(\sigma^2_c+\sigma^2_e)$.

Within-herds, years eliminated, analysis. In this analysis, the model for x_{jk} is taken as

$$x_{jk} = \mu + \underset{\text{(year)}}{s_k} + \underset{\text{(cow)}}{c_j} + \underset{\text{(error)}}{e_{jk}}.$$

By Method 3 of Henderson *(2)*, the year effects, treated as "fixed," were estimated and eliminated from the data before estimating the variance components σ^2_c and σ^2_e. Then, repeatability was again taken as $\sigma^2_c/(\sigma^2_c+\sigma^2_e)$.

RESULTS AND INTERPRETATIONS

Between-herd analysis. This analysis estimated variance components for herd, year, herd-by-year, cow, and residual error effects, the latter being within-cow variance after eliminating other effects. The results are shown (Table 4).

TABLE 4

Estimates of variance components on a between-herd basis

Variance component	Estimate	Per cent
Herd	2,372	29
Year	14	0
Herd × year	607	7
Cow (between-cow)	2,948	36
Error (within-cow)	2,308	28
Total	8,249	100

It will be noted that the year component is almost negligible, most of the "year" effects having gone into the herd × year component. In other words, in the four years under study (1951/52 to 1954/55), there was no over-all effect for the whole country, of a particular set of seasonal or climatic conditions, on the production of the herds in the sample. There was, however, a considerable variation in herd production level from year to year, thus making the herd × year component much greater than the year component. The conclusion to be drawn from this is that if a correction for "year" is to be made, it should be on an individual herd basis if it is to be effective. This is the position considered in the third analysis.

The sum of the "cow" plus "herd" components, expressed as a percentage of the total variance, gives a repeatability estimate of 0.65. This is an over-all estimate for cows in general and, as such, is comparable with the estimate obtained in the second of the two earlier analyses described, i.e., 0.61. One would expect the present estimate to be slightly higher, since it is based on production records from four years only, fewer than in the earlier work.

This estimate of repeatability may be interpreted as follows: If a cow's first record is 100 lb. of fat above the New Zealand average, the best estimate for her future lifetime production is 65 lb. of fat above the New Zealand average, provided she continues to be tested in the same herd.

Within-herds, years as replications. In treating years as replications, the effects of year on production records are virtually neglected. Only two variance components were estimated, one for cows (between-cows) and one for error (within-cows). Table 6 shows results for individual herds, and the pooled estimates obtained from grouping all herds together are shown (Table 5).

TABLE 5

Pooled estimates of within-herd variance components, neglecting year effects

Variance component	Estimate	Per cent
Cow (between-cow)	2,792	49
Error (within-cow)	2,929	51
Total	5,721	100

In this case, repeatability is estimated by expressing the "cow" component as a percentage of the total variance, viz., 0.49. This estimate is comparable with the figure obtained from the first of the earlier analyses described, where repeatability was estimated on a within-herd basis and no account was taken of "year." The estimate of 0.40 obtained from this earlier work is lower than the present estimate, again probably because considerably more than four years involved in the records used for the earlier work.

Because of the different approach used in obtaining each of the estimates, a different interpretation must be placed on each result. An estimate based on a pooling of separate estimates for each herd, and neglecting year, may be interpreted as follows: If a cow's first record is 100 lb. above *the general level of the herd (regardless of year)*, the best estimate of her future lifetime production is 49 lb. of fat above the general level of the herd she is in.

This figure of 0.49 is taken from the pooled estimates (Table 5). Values for individual herds vary considerably (see Table 6); these herd estimates are based on a much smaller number of cows and lactations than the pooled estimates; a range of 43 cows with 97 lactations to 125 cows with 295 lactations, compared with 2,436 cows with 5,557 lactations, and it can only be concluded that, on this basis, the standard errors of the individual herd estimates would be fairly large.

Within-herd analysis taking year effects into account. This analysis estimates the "year" effects for each herd separately and, after eliminating them, estimates "cow" (between-cow) and "error" (within-cow) variance components. In eliminating the year effect for the herd from each cow's record, we are virtually expressing her record as a deviation from the herd average (fluctuations in the herd average are largely "year" effects).

The analysis can, therefore, be regarded as estimating the repeatability of dairy-cow records when they are expressed as a deviation from the contemporary herd average. For most of the herds, this estimate was higher than the previous one, where no attempt had been made to eliminate year effects (see Table 6). In some herds this increase was quite considerable and, as would be expected, it was greater for herds whose average fat production had varied considerably

TABLE 6

Repeatability estimates for individual herds, from two separate analyses

Herd	Repeatability estimate		Herd	Repeatability estimate	
	Years as replications (Analysis 2)	Year effects eliminated (Analysis 3)		Years as replications (Analysis 2)	Year effects eliminated (Analysis 3)
1	0.26	0.48	16	0.70	0.76
2	0.54	0.65	17	0.70	0.75
3	0.76	0.90	18	0.54	0.70
4	0.32	0.78	19	0.60	0.63
5	0.46	0.90	20	0.39	0.45
6	0.64	0.66	21	0.45	0.46
7	0.36	0.38	22	0.59	0.71
8	0.15	0.16	23	0.72	0.72
9	0.53	0.83	24	0.60	0.73
10	0.29	0.65	25	0.47	0.55
11	0.05	0.59	26	0.65	0.62
12	0.36	0.57	27	0.53	0.55
13	0.33	0.43	28	0.48	0.30
14	0.46	0.94	29	0.47	0.53
15	0.39	0.56	30	0.53	0.67

over the four years, than for herds that had been fairly stable. Pooled estimates of the variance components for this analysis are shown (Table 7).

The cow component is 61% of the total variance and, hence, the repeatability estimate from this analysis is 0.61. Thus, by expressing a cow's record as a deviation from the herd average, in the year the record was made and not in relation to the general level of the herd over all years (as in the preceding analysis), we increase the repeatability of her record by 0.12, or approximately 25%.

The interpretation of this estimate of 0.61 for repeatability is that if a cow's first record, expressed as a deviation from the contemporary herd average, is +100 lb. fat, her average future lifetime production is best estimated as +61 lb. fat, when each of her records is expressed as a deviation from the herd average in the same year.

SUMMARY

Three different approaches were used to estimate repeatability and, correspondingly, three different interpretations are placed on the results. In the first analysis, where a herd variance component was taken into account, year effects were considered to be random, and found to be negligible—the herd-by-

TABLE 7

Pooled estimates of within-herd variance components, when year effects for each herd have been estimated and eliminated

Variance component	Estimate	Per cent
Cow (between-cow)	3,224	61
Error (within-cow)	2,030	39
Total	5,254	100

year effects absorbing all the year variations. In this analysis, repeatability (0.65) is applicable to any cow, relative to an over-all average for the whole country, and as such is not very useful.

In the first of the within-herds analyses, years were treated as mere replications and repeatability (0.49) is relative to a general herd level, regardless of year.

In the final analysis, year effects were eliminated, and repeatability (0.61) then applies to the deviation of a cow's records from her herd average in the same year. The increase from 0.49 to 0.61, when yearly effects are taken into account, indicates the importance of the within-herd, year-to-year variation, when considering repeatability estimates.

Within-herd estimates of repeatability obtained here are higher than earlier estimates, probably due to the fact that only four years are represented. It is probable that the decrease in correlations as the time interval increases (shown in Table 1), is due to long-term effects which are not likely to be directly associated with a cow's genetic make-up for butterfat production. Therefore, in using repeatability to estimate the "true producing ability" of a cow in a particular herd, there is valid reason for using the higher values based on the short-term analysis.

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SENSITIVITY OF BLOOD PLASMA AND LIVER VITAMIN A CONCENTRATIONS AND VITAMIN A DEPLETION TIME TO CAROTENE AND/OR VITAMIN A INTAKE [1]

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Holstein calves, partially depleted of vitamin A stores, were daily fed 12 to 540 γ of carotene per lb. live weight and/or 2.0 to 62.5 γ of vitamin A for either 12 or 16 wk. Hereford steers from native grass pasture were fed 5.8 to 116.0 γ of vitamin A for 40 wk. Vitamin A depletion time was more sensitive than plasma vitamin A at intakes of greater than 110 γ carotene and 18 γ of vitamin A, and more sensitive than log (liver vitamin A) at intakes of greater than 126 γ carotene and 22 γ vitamin A. Log (liver vitamin A) was more sensitive than plasma vitamin A. *Editor.*

Blood plasma vitamin A concentrations of cattle have been reported to increase at diminishing rates with an increase in intake of carotene and/or vitamin A; whereas, liver vitamin A concentrations and vitamin A depletion time increased at essentially constant rates *(4-7, 9, 12)*. This has resulted in the generally accepted belief *(1)* that liver vitamin A concentrations were more indicative of carotene and/or vitamin A intake than blood plasma vitamin A concentrations. The present study was undertaken to determine the relative sensitivity of blood plasma and liver vitamin A concentrations and vitamin A depletion time for reflecting carotene and/or vitamin A intake in cattle.

EXPERIMENTAL PROCEDURE

Animals. Data from five experiments, four conducted at the Storrs (Conn.) Station and one at the Colorado Station, were used in this study. In the first (I) experiment *(11)*, 40 male Holstein calves, 63 days of age, were depleted of

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their vitamin A stores until the blood plasma vitamin A level decreased to 10 γ/100 ml. or less. They were then fed one of five levels of carotene, 12, 18, 24, 30, or 36 γ, from alfalfa, or one of five levels of vitamin A, 2, 4, 6, 8, or 10 γ, from a dry stabilized carrier, daily per pound of live weight per calf. Upon completion of 16 consecutive seven-day vitamin supplement feeding periods, each calf was slaughtered.

In the second (II) experiment *(12)*, 48 Holstein male calves of similar age and vitamin A depletion status as in Experiment I, were fed one of three levels of carotene, 60, 180, or 540 γ from alfalfa, or one of three levels of vitamin A, 5.5, 10.8, or 21.0 γ, from a dry stabilized carrier. After the completion of 12 consecutive seven-day supplement feeding periods, 24 of the calves were slaughtered to estimate liver vitamin A stores. The remaining 24 calves were maintained on a vitamin A depletion ration, without carotene or vitamin A supplementation, until each calf's blood plasma vitamin A level decreased to less than 4.0 γ/100 ml. for two consecutive seven-day periods, to observe vitamin A depletion time *(7)*. For purposes of this study, blood plasma vitamin A and liver vitamin A values from the 24 calves that were slaughtered, and vitamin A depletion times from the 24 calves not slaughtered, were used.

In the third (III) experiment *(13)*, 32 Holstein male calves of similar age and vitamin A depletion status as in Experiments I and II were fed one of two levels of carotene, 60 or 540 γ from alfalfa without or with the addition of one of three antioxidants, N,N′-diphenyl-para-phenylenediamine, 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline, or 2,5-ditertiarybutylhydroquinone, fed at the rate of 0.01% of the ration for 12 consecutive seven-day supplement feeding periods. The calves were then fed a vitamin A depletion ration which, in one-half of the calves, was supplemented with the antioxidant previously fed. This regimen was maintained until each calf's blood plasma vitamin A level had decreased to less than 4.0 γ/100 ml. for two consecutive seven-day periods, to observe vitamin A depletion time.

In the fourth (IV) experiment *(4)*, 48 Holstein male calves, partially depleted of their vitamin A stores, were fed simultaneously at 63 days of age, one of four levels of carotene, 20, 50, 125, or 312.5 γ from alfalfa, and one of four levels of vitamin A, 4, 10, 25, or 62.5 γ, from a dry stabilized carrier. After 16 consecutive seven-day vitamin supplement feeding periods, each calf was fed a vitamin A depletion ration, without carotene and vitamin A supplementation, for four consecutive seven-day periods. At the end of this time, each calf was slaughtered to determine liver vitamin A stores. The blood plasma values obtained during this period were used to estimate vitamin A depletion time *(15)*.

In the fifth (V) experiment *(6)*, conducted at the Colorado Station, 114 Hereford steers, approximately eight months of age and previously on native grass pasture, were divided into six lots of 19 animals each. Lot 1 was slaughtered to determine the initial vitamin A status, and Lots 2 through 6 were fed one of five levels of vitamin A, 0.0, 5.9, 23.3, 46.7, or 116.0 γ, from fish oil for ten 28-day periods. Nine steers from each lot were slaughtered after 166 days

on experiment, so that at the completion of the experiment, 280 days, only ten steers per lot remained. For the purposes of this study, data at the termination of the experiment from Lots 3 through 6 were used.

Criteria and analyses. The amounts of carotene and/or vitamin A supplements fed were adjusted to live weight at successive seven-day intervals for Experiments I through IV, and at 28-day intervals for Experiment V. Blood samples for the determination of plasma vitamin A concentrations by the Kimble procedure *(8)* were taken prior to slaughter, and liver samples for vitamin A concentration by the Davies method *(3)*, upon slaughter. Vitamin A depletion time, defined as the number of weeks for the blood plasma vitamin A level to decrease to 4.0 γ/100 ml., upon feeding a vitamin A depletion ration *(7)*, was actually observed for calves in Experiments II and III, but was calculated *(15)* for calves in Experiment IV. Standard statistical procedures *(2, 14)* were used in the analyses of the data.

RESULTS AND DISCUSSION

Table 1 contains, by experiments, the mean plasma vitamin A and mean log (liver vitamin A) concentrations and the mean vitamin A depletion times, the standard deviation per animal for these three criteria, and the standard devia-

TABLE 1

The mean, standard deviation, and coefficient of variation of plasma vitamin A, log (liver vitamin A), and vitamin A depletion time for five experiments

	Experiments				
	I	II	III	IV	V
Literature reference	*(12)*	*(13)*	*(14)*	*(4)*	*(6)*
			(γ/lb live weight/day)		
Intake range					
Carotene	12–36	60–540	60–540	20–312.5	....
Vitamin A	2.0–10.0	5.5–21.0	.....	4.0– 62.5	5.8–116.0
Duration of supplement feeding *(wk.)*	16	12	12	16	40
No. of animals per intake group *(n)*	4	4	16	12	10
			(γ/100 ml.)		
Plasma vitamin A					
Mean $(\bar{y})$	10.9	23.5	23.8	14.8[a]	47.5
Standard deviation (s_y)	2.3	2.8	3.9	4.0	7.3
Coefficient of variation (C.V.)	21.1	11.9	16.4	27.0	15.4
			(log [γ/100 g.])		
Liver vitamin A					
Mean $(\bar{y})$	1.74	2.72	.....	2.58[a]	3.24
Standard deviation (s_y)	0.19	0.17	.....	0.31	0.19
Coefficient of variation (C.V.)	10.9	6.2	.....	12.0	5.9
			(wk.)		
Vitamin A depletion time					
Mean $(\bar{y})$	.....	6.9	8.0	9.0	.....
Standard deviation (s_y)	.....	1.0	2.1	1.4	.....
Coefficient of variation (C.V.)	.....	14.5	26.2	15.6	.....

[a] Values after four weeks' depletion following supplement feeding period.

tions as a percentage of their respective means, coefficients of variation. In Experiment IV, the plasma and log (liver vitamin A) concentrations represented values after four weeks of vitamin A depletion; thus, the relatively low magnitude of their values. Since the variances of liver vitamin A concentrations were greater at the higher carotene and/or vitamin A intakes than at the lower, liver vitamin A values were transformed to their respective logarithms (Base 10). This was for the purpose of maintaining essentially constant variability in this response criterion for all carotene and/or vitamin A intakes, so that the assumption of common variance among intake groups would be met *(2, 14)*.

Across all experiments, the average coefficient of variation, weighted for the number of animals in each experiment, was 19.4% for plasma vitamin A, 9.2% for log (liver vitamin A), and 18.6% for vitamin A depletion time. Thus, log (liver vitamin A) exhibited the smallest experimental error as a percentage of its mean of the three measurements, with a relatively small difference between plasma vitamin A and vitamin A depletion time.

Although a small experimental error is highly desirable, decisions as to the choice of a criterion to measure response to a particular treatment (i.e., carotene and/or vitamin A intake in this study) are generally based on the magnitude of response of the criterion, as well as on some estimate of its experimental error. In such decisions, the main consideration is to obtain maximum treatment response as well as minimum experimental error. Thus, if a decrease in experimental error is accompanied by a proportionally smaller treatment difference, *little is to be gained by changing from one criterion to another.* Recently, Mandel and Stiehler *(10)* have proposed the use of the ratio of the rate of change of the criterion with respect to treatment, dy/dx, to the standard deviation of the criterion, s_y, as a quantitative measure of merit between criteria. This ratio, termed sensitivity, will remain constant for all values of a treatment in which the response of a criterion remains linear, but will vary when the responses are not linear. Unlike the standard deviation and coefficient of variation, a unique property of this ratio is that it is invariant with respect to any functional transformation of a criterion. Also, as long as the treatment (i.e., carotene and/or vitamin A intake in this study) is expressed in the same scale and within the same limits, comparisons as well as tests of significance between criteria can readily be made. In the present study, this sensitivity ratio, $(dy/dx)/(s_y)$, or in the more restricted case in which essentially linear responses of the criterion on treatment are found, the ratio $(b)/(s_y)$, will be used for comparative purposes.

The linear slopes, b, of the respective criteria on carotene or vitamin A intake, with their 95% confidence limits, simple correlations, r, of the respective criteria and intake, and the ratio of the linear slopes to the standard deviations of the respective criteria, b/s_y, sensitivity, are given in Table 2. As was pointed out previously, plasma vitamin A concentrations increased at diminishing rates with increases in carotene and/or vitamin A intake. Therefore, transformation of the carotene and vitamin A intakes to their respective logarithms (Base 10) was employed to insure constant (linear) responses in plasma vitamin A concentrations over the range of intakes studied.

TABLE 2

Sensitivity of three criteria to carotene and/or vitamin A intake, based on linear slopes of response over the standard deviation of the response criterion

	Criteria									
	Plasma vitamin A in γ/100 ml.			Liver vitamin A in log (γ/100 g.)			Vitamin A depletion time in wk.			
	Linear slope on log intake and 95% confidence limits of slope	Simple correlation between intake and response	Sensitivity[a]	Linear slope on log intake and 95% confidence limits of slope	Simple correlation between intake and response	Sensitivity[a]	Linear slope on actual intake and 95% confidence limits of slope	Simple correlation between intake and response	Sensitivity[a]	Sensitivity as a function of log intake[b]
	(b)	*(r)*	*(S)*	*(b)*	*(r)*	*(S)*	*(b)*	*(r)*	*(S)*	
Carotene fed										
Expt. I	14.4 8.1 to 20.7	0.92	6.3	2.00 1.49 to 2.51	0.99	10.8	.....	.....	.....	.....
II	7.9 3.5 to 12.3	0.98	2.8	0.83 0.55 to 1.11	1.00	4.8	0.0166 0.0086 to 0.0196	1.00	0.0	2.3 to 20.6
III	7.2 4.2 to 10.2	.....[c]	1.8	.....	.....	.....	0.0149 0.0116 to 0.0182	.....[c]	0.0	1.0 to 8.8
Vitamin A fed										
Expt. I	19.8 15.6 to 24.0	0.94	8.6	2.40 2.05 to 2.75	0.99	12.9	.....	.....	.....	.....
II	24.3 17.0 to 31.6	1.00	8.7	1.94 1.49 to 2.39	0.99	11.1	0.4955 0.3968 to 0.5942	0.99	0.5	6.3 to 23.9
V	22.2 17.3 to 27.1	0.96	3.0	1.56 1.43 to 1.69	0.99	8.2	.....	.....	.....	.....
Carotene and vitamin A fed										
Expt. IV										
Carotene	6.9 4.2 to 9.6	0.98	1.7	0.72 0.51 to 0.93	0.98	2.3	0.0107 0.0070 to 0.0144	0.94	0.0	0.3 to 5.5
Vitamin A	14.2 11.5 to 16.9	0.97	3.6	1.36 1.15 to 1.57	0.98	4.3	0.1239 0.1049 to 0.1429	0.98	0.1	0.8 to 12.7

[a] Sensitivity, S, equals slope, b, divided by the standard deviation of the response, s_y.

[b] $S = (\frac{b}{s_y})\ 2.30x$, where x = intake in actual units (see Statistical Appendix following this paper). S values are for the lowest and highest intakes, respectively.

[c] Correlation equals 1.0, since only two levels of intake were fed; thus, no residual variation existed.

Since it was assumed that liver vitamin A concentrations and vitamin A depletion time increase at essentially constant rates with increases in carotene and/or vitamin A intake, in the initial calculations no transformation of the intake values was employed. Although a satisfactory relationship for vitamin A depletion time and intake was found, the variation associated with liver vitamin A concentrations increased with increasing intake. To insure essentially equal variability in liver vitamin A concentrations across all intake levels, as well as to maintain the assumed linear relationships of the actual liver vitamin A concentrations on actual intake, both liver vitamin A concentrations and carotene or vitamin A intakes were transformed to their respective logarithms. This resulted in homogenous variability across intake levels as determined by Bartlett's test of homogeneity of variances *(14)*, and in essentially linear relationships of the transformed variables.

From the sensitivity ratios (Table 2), it was clearly evident that log (liver vitamin A) was considerably more sensitive to carotene and/or vitmain A intake than plasma vitamin A. Since the sensitivity ratios of vitamin A depletion time (second-to-last column, Table 2) were measured in terms of γ intake and not log (γ intake), no valid comparisons of the over-all sensitivities between depletion time and plasma and log (liver vitamin A) could be made.

The response of vitamin A depletion time was essentially linear over the entire range of intakes; whereas, those for plasma vitamin A and log (liver vitamin A) diminished with increasing intake, but were essentially linear against log (intake). Therefore, sensitivity of depletion time would be expected to increase with log (intake). In the last column of Table 2, the sensitivity ratios of the preceding column were multiplied by $2.30x$, in which x represents the actual intake (see Statistical Appendix to this paper, for explanation and derivation), for the lowest and highest intakes, respectively, to give a range of sensitivity ratios comparable to those for plasma and liver vitamin A. At the lower intakes, depletion time was not as sensitive as plasma or log (liver vitamin A) to intake; but as the intake increased, it became considerably more sensitive than either plasma or log (liver vitamin A). It was calculated that, at intakes of greater than 110 γ of carotene and 18 γ of vitamin A, depletion time was more sensitive to intake than plasma vitamin A. Similar values in the case of log (liver vitamin A) were 126 γ carotene and 22 γ vitamin A.

All three criteria, under the conditions of the experiments reported herein, were satisfactory for the detection of statistically significant differences between the wider intakes of carotene and/or vitamin A, or statistically significant linear slopes of the respective criteria on intake *(4, 6, 11-13)*. In terms of application, however, to obtain a specified statistically significant difference between two carotene or vitamin A intakes expressed as logarithms, more animals per intake group (replications) are needed when using plasma vitamin A as the criterion than when using log (liver vitamin A). In the case of vitamin A depletion time, at lower intakes of carotene or vitamin A, greater replication is needed than for plasma or liver vitamin A; whereas, at the higher intake levels, the opposite is true. For purposes of illustrating the number of replications needed to deter-

mine a specified statistically significant difference, when using the three criteria, consider the data from Experiment II *(12)*. The difference in the blood plasma vitamin A between the middle carotene intake, 180 γ, and the lowest, 60 γ, was 4.2, the difference for log (liver vitamin A), 0.400, and for vitamin A depletion time, 2.5 [see Tables 2 and 3 of Reference *(12)*]. Dividing these values by their respective experiment means, 23.5, 2.718, and 6.9 (from Table 1, this paper), and multiplying each quotient by 100, gives 17.9, 14.7, and 36.2%, respectively, for the difference as a percentage of the mean response. The differences as percentages of their means and the average coefficients of variation as previously cited, 19.4% for plasma vitamin A, 9.2% for log (liver vitamin A), and 18.6% for vitamin A depletion time, were referred to Table 2.1 (for a one-tailed test with significance at the 5% level and probability 80%) of Cochran and Cox *(2)*. This provided an estimate of the number of replications required for a given probability of obtaining a significant result. It was found that when using plasma vitamin A as the criterion to measure carotene intake, approximately 16 replications were required, when using log (liver vitamin A) six replications, and when using vitamin A depletion time, five replications. By following the procedures as outlined by Cochran and Cox *(2)*, and the statistics contained herein, some indication of the number of replications needed can be obtained for any specified intake of carotene or vitamin A for the three criteria studied.

Some precaution is necessary in the use of the statistics reported herein. It is known (Tables 1 and 2) that variation existed in the responses as well as in the experimental errors, for any one particular criterion among experiments conducted at a single experiment station (i.e., the Storrs Station), and possibly greater variation from experiment station to experiment station (i.e., the Storrs Station data *versus* the Colorado Station data). Therefore, such statistics are at best only guides, and it is preferable to have for the preceding calculations average responses and experimental errors for the particular conditions under which the experiment is to be undertaken.

Also, it should be pointed out that the data contained herein were from animals partially depleted of their vitamin A stores, Experiments I through IV, or from animals fed the vitamin A supplement for a sufficiently long period (40 wk.) so that the animal's vitamin A status apparently stabilized, Experiment V. Under experimental conditions other than these, such as a short carotene and/or vitamin A supplement feeding period, without partial vitamin A depletion, different results might have been obtained. Until data under such conditions are collected and quantitatively analyzed, the results of the study reported herein can not have general application.

SUMMARY

Blood plasma vitamin A concentrations and liver vitamin A concentrations obtained at slaughter, upon the completion of a carotene and/or vitamin A supplement feeding period and vitamin A depletion time (the weeks required for the blood plasma vitamin A level to decrease to 4.0 γ/100 ml., when only a

vitamin A depletion ration was fed upon completion of the supplement feeding), were obtained from five experiments. In four of these, Holstein calves previously partially depleted of their vitamin A stores were fed carotene intakes ranging from 12 γ to 540 γ per lb. live weight per day and/or vitamin A intakes ranging from 2.0 γ to 62.5 γ for periods of either 12 or 16 wk. In the fifth experiment, Hereford steers previously grazing native grass pasture were fed intakes of vitamin A from 5.8 to 116.0 γ for a period of 40 wk. The following results were obtained:

(1) Average coefficients of variation across all experiments were 19.4% for plasma vitamin A, 9.2% for log (liver vitamin A), and 18.6% for vitamin A depletion time. Thus, log (liver vitamin A) exhibited a smaller experimental error as a percentage of its mean than either plasma vitamin A or vitamin A depletion time.

(2) Average sensitivities of these criteria to carotene or vitamin A intake, which was defined as the ratio of the linear slope of the response criteria on intake to the standard deviation of the criterion, Mandel and Stiehler *(10)*, was 4.6 for plasma vitamin A on log (intake), 7.8 for log (liver vitamin A) on log (intake), and 0.1 for vitamin A depletion time on actual intake. Calculation of the sensitivity of vitamin A depletion time in terms of log (intake) gave a range of sensitivities between 0.3 and 23.9, which increased with the intake level. Depletion time was calculated to become more sensitive than plasma vitamin A at carotene intakes of greater than 110 γ and vitamin A intakes of greater than 18 γ, and more sensitive than log (liver vitamin A) at carotene intakes of greater than 126 γ and at vitamin A intakes of greater than 22 γ.

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STATISTICAL APPENDIX

Sensitivity, as used in this paper, is defined as $\frac{dy}{dx}/\sigma_y$ where x represents intake and y response. Thus, sensitivity is the increase in response corresponding to a one-unit increase in intake, the response being expressed in units of its standard deviation. It results from this definition that a change of scale in the response will not alter the sensitivity, because this change will be reflected in the standard deviation in exactly the same proportion. On the other hand, a change of scale in intake will obviously affect the sensitivity.

Suppose that we subject the intake x to a logarithmic transformation. We then have:

a) in the original scale:

$$\text{Sensitivity} = \frac{dy}{dx} / \sigma_y \tag{1}$$

b) in the transformed scale:

$$\text{Sensitivity} = \frac{dy}{d(log_{10}x)} / \sigma_y \tag{2}$$

In order to relate these two quantities, we must find the relation between the differential dx and $d(log_{10}x)$. From differential calculus it is known that:

$$d(log_{10}x) = \frac{1}{x}(log_{10}e)dx = \frac{0.4343}{x}dx = \frac{1}{2.30x}dx. \tag{3}$$

From (3) it follows that:

$$\text{Sensitivity}_{(2)} = \frac{dy}{\frac{1}{2.30x}dx} / \sigma_y = (2.30x)\frac{dy}{dx} / \sigma_y$$

Consequently,

$$\text{Sensitivity}_{(2)} = (2.30x)\ (\text{sensitivity}_{(1)}). \tag{4}$$

Applying these results to the study of vitamin A depletion time, we see that if the sensitivity of this criterion is found to be constant over the entire range of intake values, when the latter are expressed in the original units (x), the sensitivity will no longer be constant, when the intake x is measured on a logarithmic scale. In the latter case, because of Equation (4), the sensitivity will increase with x. Since both plasma vitamin A and liver vitamin A are studied as functions of the logarithm of intake, a comparison of the sensitivity of vitamin A depletion time with the sensitivities of these criteria requires that its sensitivity be also computed in terms of the logarithm of intake. It is for this reason that the last column of Table 2 was computed.

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VOLATILE AND NONVOLATILE SULFHYDRYL CONTENT OF HEATED MILK AND MILK PRODUCTS[1]

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Simultaneous determinations were made of the volatile -SH groups (measured as hydrogen sulfide) and the nonvolatile -SH groups (expressed as thiamine disulfide [TDS] values) produced by the heating of milk, buttermilk, whey, and cream. Heating at 90° C. momentarily, yielded average TDS values of 21.0 for milk, 17.4 for whey, 22.8 for cream, and 22.9 for buttermilk. Heating milk, cream, buttermilk, and whey to 90° C. for 30 min. yielded hydrogen sulfide per liter of 433, 535, 596 γ, respectively. For all products, the TDS values decreased 20 to 22% as a result of liberation of the H_2S which, for milk, amounted to a decrease of 1.0 mg. TDS value per 0.1 mg. of hydrogen sulfide removed. Addition of copper, calcium chloride, or disodium phosphate, or low-temperature forewarming and condensing, affected the concentration of heat produced -SH groups. *Editor.*

One significant manifestation of high-temperature heating of milk is the formation of highly reactive sulfhydryl (-SH) groups. In recent years, considerable attention has been given to detecting and measuring these groups, and to the factors affecting their concentration *(2, 3, 5-8, 11, 12, 14-17)*. In this connection, the terms "reactive" and "active" are used, to differentiate the heat-revealed -SH groups from the total -SH concentration in unheated milk and measured by the iodosobenzoate method *(11, 12)*.

Among the methods used for determining the active -SH groups in heated milk have been those measuring hydrogen sulfide (H_2S) *(15)*, and thiamine disulfide–reducing capacity (TDS method) *(7)*. The former reveals only the volatile portion of the heat-revealed -SH groups; whereas, the TDS values are affected by all -SH compounds which are created or revealed by the heat treatment, including H_2S *(7)*. However, Harland and Ashworth indicate that the effect of H_2S on the TDS values is slight.

This study was conducted to determine the total -SH group reducing capacity of milk following heating, and was designed to permit the simultaneous determinations of the H_2S and TDS values.

EXPERIMENTAL PROCEDURES

The approach to the problem of simultaneous measurement of the volatile and the nonvolatile -SH groups in heated milk involved the adoption of procedures utilized in previous studies, with certain modifications. The "volatile" portion of the active -SH groups was measured and expressed as H_2S. The pro-

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cedure for collection of the H_2S was essentially that of Townley and Gould *(15)*, and the methylene blue method of Fogo and Popowsky *(4)* was used to measure the H_2S concentration.

The measurement of the so-called "nonvolatile" portion of the heat-revealed -SH compounds was by the thiamine disulfide (TDS) method of Harland and Ashworth *(7)*, slightly modified. It is granted that the H_2S, per se, contributes in a small degree to the TDS values. However, under any set of conditions, it was accepted that a rather clear-cut differentiation would be made between the -SH groups in the gaseous state and those remaining in the milk system. It is recognized, too, that reducing materials in milk other than -SH groups may contribute to the TDS values. Therefore, the TDS values are used arbitrarily to represent the nonvolatile -SH results. These nonvolatile results are expressed as mg. cysteine · HCl/l of milk.

The system used for heating the milk and collecting the volatile -SH compounds consisted of the following steps: Nitrogen was passed through alkaline pyrogallol and into a closed one-liter Pyrex test tube which contained the milk being heated. The Pyrex tube was suspended in a thermostatically controlled water-bath. The nitrogen which aspirated and agitated the milk during the heat treatment passed through an Erlenmeyer flask trap and into two Pyrex glass receivers arranged in series. These receivers (2.5 cm. × 50 cm.) contained alkaline zinc acetate solution to trap the H_2S. The nitrogen carrying the H_2S was dispersed finely into the acetate solution through 12-mm. medium fritted-glass cylinders, suspended near the bottom of the receivers. Preliminary trials revealed complete recovery of the liberated H_2S in the first receiver.

To determine the amount of sulfide by the modification of the Fogo and Popowsky method *(4)*, the zinc acetate solution (100 ml. in each receiver) was transferred quantitatively to a 250-ml. volumetric flask, the receiver was rinsed, and the color developed with 25 ml. of *p*-amino dimethylaniline solution (0.5 g. in 500 ml. 5.5 *M* HCl). Five ml. of ferric chloride solution (0.023 *M* solution in 1.2 *M* HCl) was added. After ten minutes, the volume was adjusted to 250 ml. with distilled water, the mixture allowed to stand for 20 min., and the color intensity determined by means of a Model DU Beckman Spectrophotometer at a wave length of 675 mμ. Comparison was made to the standard curve prepared by a modification of the Lachelle procedure *(10)*, utilizing Bureau of Standard Steel, BOH, 14c, containing 0.03% sulfur.

The thiamine disulfide was prepared according to Harland and Ashworth *(7)*. The oxidizing agent (quinine sulfate), and the thiamine-HCl solutions were prepared as recommended by Official Methods *(1)*, and stored in dark bottles. The standard curve was verified each time new alcohol or thiamine disulfide was used.

The thiamine disulfide–reducing materials in milk were measured as recommended by Harland and Ashworth *(7)*, except that the one ml. of trichloroacetic acid filtrate was made to a volume of 50 ml. with distilled water and two ml. of the filtered material were used for the estimation of the amount of thiamine present.

A known amount of standard thiamine solution served as a control when the milk samples were analyzed. Blank values for the milk were obtained by adding the trichloroacetic acid before the thiamine disulfide (in the case of heated milk), or after the incubation period (in the case of raw milk). The observations of Harland and Ashworth were corroborated, in that raw milk did not contain any thiamine disulfide–reducing materials, nor was there further reduction of thiamine disulfide after the addition of the acid.

Unless otherwise stated, carefully selected, fresh mixed-herd milk was used. The milk was heated in 500-ml. lots in the one-liter Pyrex test tube. Three drops of a Dow Corning antifoam agent (silicone product) were added to the milk prior to heating, to prevent foaming. As previously noted, agitation was provided by bubbling nitrogen through the sample during heating, and during each subsequent aspiration period.

The milk was brought to the highest desired temperature, 90° C., within approximately eight minutes, and the temperature controlled to an accuracy of $\pm$ 0.5° C. After being heated, the milk was transferred in the test tube to a cold water-bath, and cooled to a temperature below 75° C. within one minute. Aspiration which followed heating was conducted at approximately 15° C.

RESULTS

One of the first objectives of this problem was to adopt a method whereby the H_2S produced by heating milk could be removed from the sample completely and quickly. Previous observations *(15)* indicated that the use of an aspiration procedure alone was slow and gave low recoveries of the hydrogen sulfide. Therefore, a study was made of the aspiration procedure used in this study, as applied to normal milk and to milk acidified with a strong acid. It was thought that an acid treatment prior to aspiration might accelerate greatly the release of the hydrogen sulfide.

In this experiment, raw milk was divided into four lots: Lot 1—served as the control and was aspirated for 30 min.; Lot 2—was acidified to pH 2.5 with HCl and then aspirated; Lot 3—was acidified to pH 2.5, heated to 90° C. for 30 min., and cooled; Lot 4—was heated to 90° C. for 30 min., cooled, then acidified to pH 2.5. Lots 3 and 4 were aspirated throughout the treatment period and for the given time thereafter. Results are presented (Table 1).

The results reveal that a slight but measurable amount of H_2S was produced as a result of the drastic acidification of raw milk, and that this amount was increased only slightly, i.e., from 30 to 63 γ, by the heat treatment.

The results in the table reveal, also, that acidification adversely affects the ability of milk to yield volatile sulfide on subsequent heating. For example, only a small amount of H_2S was produced in the milk heated following acidification (Lot 3), as contrasted to the quantity produced when the milk was acidified after heating (Lot 4). In the latter case, the major share of the H_2S had been produced before the acidification occurred, i.e., during the heat treatment itself.

TABLE 1

Effect of acidification on the release of hydrogen sulfide from heated and nonheated milk (av. of 3 trials)

Lot No.	Treatment of sample		Aspiration time	Hydrogen sulfide
			(min.)	*(γ/l)*
1	Raw milk control		30	0
2	Raw milk acidified—pH 2.5		30	30
			30	0
		Total	60	30
3	Raw milk acidified to pH 2.5, heated at 90° C. for 30 min., and cooled		30	63
			30	0
		Total	60	63
4	Raw milk heated at 90° C. for 30 min., cooled, acidified to pH 2.5		30	350
			30	60
			30	24
			30	0
		Total	120	434

Based on these preliminary observations, additional experiments were conducted in which milk was subjected to heat treatments of 90° C. for 10 min. and for 30 min. Determinations were made of the volatile and the nonvolatile -SH groups of the heated milk, with and without acidification following heating. Results are shown (Table 2).

These results reveal that 90 min. of aspiration after heating permitted complete recovery of the H_2S which had been produced, and that the rapidity of the recovery was only slightly improved by the acid treatment. The total H_2S recovered was not greatly different between the normal and the acid-treated series.

The volatile -SH results also reveal the importance of temperature and time of heating. Relatively small amounts of H_2S were detected by the time the 90° C. temperature was reached; whereas, the major portion of the H_2S was liberated during the holding and/or aspiration period. This is especially noticeable in the series involving a 30-min. heating period.

Prior to making the TDS determinations, a study was made of the effect of pH on the reduction of thiamine hydrochloride by cysteine hydrochloride. The results revealed that, *(a)* quantitative results were obtained at pH values of 5.2 to 6.0, and low results were obtained at pH values of 4.4 and below; *(b)* in the normal determination, the reaction mixture of milk and thiamine disulfide averages about pH 5.4, and *(c)* the acidified milk samples gave extremely low TDS values unless the mixture of milk and thiamine disulfide was buffered at about pH 5.6.

The TDS values, as cysteine · HCl, were highest at the time the 90° C. temperature was achieved, ranging from 19 to 22 mg/l, and became progressively less as the heating and/or aspiration period progressed. This indicates that the compounds responsible for the TDS values are produced before H_2S is liberated.

TABLE 2

Volatile and nonvolatile sulfhydryl content of heated milk, with and without acidification following heating

Treatment of samples		Aspiration time	Hydrogen sulfide	TDS values as cysteine-HCl
		(min.)	*(γ/l)*	*(mg/l)*
	SERIES I			
Control				
Heated to 90° C.		—	14	21.9
90° C.—10 min.		10	85	20.1
Cooled		30	38	16.6
		30	24	17.2
		30	0	16.9
	Total	100	161	
Acidified				
Heated to 90° C.		—	34	19.0
90° C.—10 min.		10	110	19.0
Cooled—acidified pH 2.5		30	10	—
		30	10	—
		30	0	—
	Total	100	164	
	SERIES II			
Control				
Heated to 90° C.		—	—	21.9
90° C.—30 min.		30	373	17.5
Cooled		30	52	17.4
		30	24	17.7
		30	0	17.6
	Total	120	449	
Acidified				
Heated to 90° C.		—	—	22.0
90° C.—30 min.		30	410	18.9
Cooled—acidified—pH 2.5		30	56	14.1[a]
		30	0	14.3[a]
		30	0	14.3[a]
	Total	120	466	

[a] It was necessary to buffer these samples at pH 5.6 for the TDS determinations, in order to obtain quantitative results. Also, difficulty in obtaining an accurate sample from the coagulated sample probably contributed to the slightly lower results in this series.

The liberation of H_2S was accompanied by decreases in the TDS values, with the major changes occurring during the first 30 min. following the reaching of 90° C. A typical change is that presented for the control sample in Series 2. In this series, the TDS values decreased from 21.9 mg/l to 17.5 mg/l over the 30 min. of heating (corresponding to an evolution of 373γ/l of H_2S), and were rather constant for the remainder of the aspiration period. The total evolution of 449γ/l of H_2S was equivalent to a loss of the TDS value of 4.3 mg/l, or an approximate molar ratio of 1:2.

Since the H_2S was removed by aspiration alone, with only a slightly longer aspirating period than was required for the acidified samples, acidification was omitted from the procedure used for the remaining experiments.

Volatile and nonvolatile -SH compounds in other products. Experiments were conducted in which rennet whey, 35% cream, and buttermilk from 35% cream

TABLE 3

Volatile and nonvolatile sulfhydryl content in heated whey, buttermilk, and cream (av. of 4 trials)

Treatment of sample		Aspiration time	Hydrogen sulfide	TDS values as cysteine-HCl
		(min.)	*(γ/l)*	*(mg/l)*
Whey				
90° C.		—	—	17.4
90° C.—30 min.		30	229	13.7
		30	48	13.9
		30	16	13.7
		30	0	13.6
	Total	120	293	
35% cream				
90° C.		—	—	22.8
90° C—30 min.		30	468	17.9
		30	52	17.4
		30	15	17.5
		30	0	17.6
	Total	120	535	
Buttermilk				
90° C.		—	—	22.9
90° C.—30 min.		30	500	17.7
		30	66	17.5
		30	30	17.6
		30	0	17.3
	Total	120	596	

were heated to 90° C. for 30 min. and the H_2S and the TDS values determined. Results are presented (Table 3).

The trends shown are, in general, similar to those which were obtained for milk. The highest TDS values were obtained with momentary heating, and subsequent liberation of the H_2S lowered the cysteine · HCl values 3.8 mg/l, 5.2 mg/l, and 5.6 mg/l for whey, cream, and buttermilk, respectively. The higher losses of the TDS values were accompanied by greater liberation of H_2S, viz., 596 γ for buttermilk as compared to 292 γ for whey. In all cases, the H_2S liberation was complete at the end of 90 min. of aspiration, and the loss in the TDS values approximated from 22 to 24%.

The results corroborated earlier findings *(4)*, in that the H_2S is in far greater concentration in heated cream and buttermilk than in whey.

Effect of copper. It has been shown that copper decreases the amount of H_2S produced when milk is heated *(6)*. A study was conducted to demonstrate the effect of copper, added as copper sulfate either before or after heating, upon both the volatile and nonvolatile -SH content. Milk was divided into three lots and treated as follows:

Lot 1—Control—heated at 90° C. for 30 min.; Lot 2—0.5 p.p.m. copper was added, milk then heated to 90° C. for 30 min.; Lot 3—milk heated to 90° C., 0.5 p.p.m. copper added, then milk held 30 min. at heating temperature.

In all cases, the aspiration time, including the 30-min. heating period, was 120 min. Results are given (Table 4).

TABLE 4

Effect of adding copper on the volatile and nonvolatile sulfhydryl content in heated milk (av. of 4 trials)

Sample	Aspiration periods	H_2S	TDS values as cysteine-HCl
Lot 1—Control	*(min.)*	*(γ/l)*	*(mg/l)*
90° C.	0	—	21.6
90° C—30 min.	30	345	18.2
Final	120	429[a]	17.4
Lot 2—0.5 p.p.m. before heating			
90° C. momentarily	0	0	17.9
90° C.—30 min.	30	122	14.0
Final	120	155[a]	14.1
Lot 3—0.5 p.p.m. at 90° C.			
90° C. momentarily	0	—	15.9
90° C.—30 min.	30	122	12.6
Final	120	165[a]	12.5

[a] Total H_2S liberated during the heating and 120 min. of aspiration.

The results reveal that copper has a marked adverse effect on both the H_2S and the TDS values. The control yielded 429 γ H_2S and TDS values of 21.6 mg. cysteine · HCl; whereas, milk to which copper was added prior to heating produced only 155 γ of H_2S, and had an initial TDS value of 17.9 mg/l.

The addition of 0.5 p.p.m. copper to the milk immediately at the time the heating temperature was reached, and after the -SH groups were formed, also reduced the -SH concentration in comparison to the control. The volatile -SH results were approximately the same, regardless of the time the copper was added. However, the TDS values were somewhat lower when the copper was added after the milk had reached 90° C., than when added prior to heating. The possibility exists that some of the copper added before heating may be made unavailable for destroying -SH groups by combining with other milk constituents.

In another series, for which detailed data are not given, milk was heated to 90° C. and cooled. Then 2.0 p.p.m. copper were added and the milk reheated to 90° C. for 30 min. No H_2S was produced during the second heat treatment. The TDS value of the milk heated to 90° C. after the copper treatment was 10.2 mg/l, and this remained essentially constant during the subsequent aspiration time of 120 min. This indicates that a portion of the nonvolatile -SH groups is rather resistant to copper destruction.

To study further the resistance of the nonvolatile portion of the -SH compounds to copper, milk was heated momentarily to 90° C., treated while hot with 1, 3, 5, and 7 p.p.m. of copper, cooled, and stored at 4° C. TDS determinations were made at 0-, 24-, and 48-hr. periods and are presented (Figure 1).

These results reveal that the destruction of the reactive nonvolatile -SH groups is directly related to concentration of copper and to storage time. The relationship of copper concentration to the nonvolatile -SH destruction at 0-time was essentially linear, and the calculated ratio of the loss of these -SH groups to concentration of copper was 1 :1.1 on a molar basis.

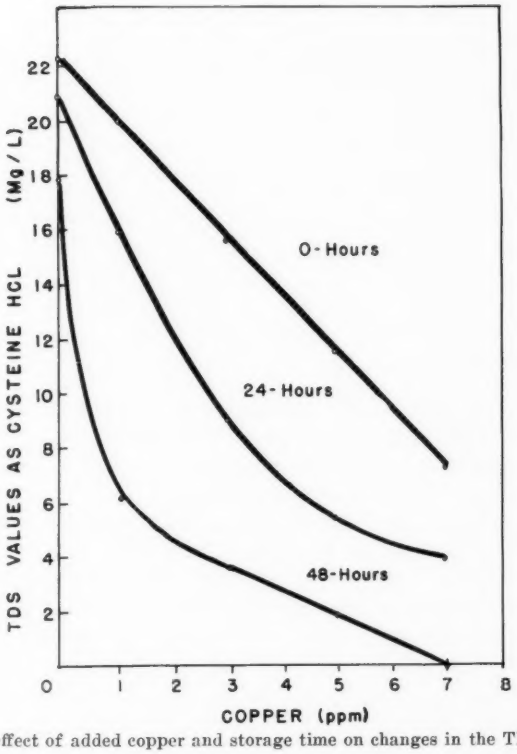

FIG. 1. The effect of added copper and storage time on changes in the TDS values of milk heated to 90° C.

The importance of the function of time in the copper destruction of the -SH groups is revealed by the results for the lots containing 7 p.p.m. of added copper. The addition of this high concentration of copper immediately reduced the TDS values 67%, but the groups contributing to the TDS values were not eliminated entirely until the 48-hr. analysis.

Calcium chloride and disodium phosphate. In preliminary work, it was noticed that samples which were somewhat heat-unstable gave lower hydrogen sulfide results than milk which was heat-stable. Therefore, trials were conducted in which 0.025% calcium chloride and 0.025% disodium phosphate were added to milk and to rennet whey; the products were heated and the -SH content determined. The results are presented (Table 5).

For the milk, the addition of either the calcium chloride or the disodium phosphate decreased the concentration of both the volatile and the nonvolatile -SH compounds, in comparison to the control. The effect was greater in the case of the calcium salt.

The effect of the calcium chloride was essentially the same on the whey as on the milk, but the addition of disodium phosphate to the whey increased the

TABLE 5

Effect of adding $CaCl_2$ and Na_2HPO_4 on the volatile and nonvolatile sulfhydryl content in heated milk and whey (av. of 4 trials)

Sample	H_2S	TDS values as cysteine-HCl At 90° C.	Final[a]
	(γ/l)	*(mg/l)*	*(mg/l)*
Milk			
Control	454	20.3	16.5
$CaCl_2$ (0.025%)	334	16.9	15.6
$NaHPO_4$ (0.025%)	390	17.0	15.4
Whey			
Control	252	17.1	13.7
$CaCl_2$ (0.025%)	206	13.9	13.8
Na_2HPO_4 (0.025%)	320	17.1	13.2

[a] Total aspiration time, 120 min.

concentration of the volatile -SH compounds in comparison to the control, and had little effect on the nonvolatile portion. As may be anticipated, the visible protein precipitation produced by the heat treatment was increased by the added calcium and decreased by the added phosphate.

Another experiment was conducted, in which milk from a commercial source was obtained which had been processed through a Zeolite bed. This treatment removed approximately 16% of the calcium. The Zeolite-treated milk yielded essentially the same concentration of -SH groups on heating as did normal milk.

Effect of preheating and/or concentration on -SH compounds produced in milk subsequently heated. Experiments were conducted to determine the effect of preheating and/or condensing at low temperatures on the formation of the reactive -SH groups.

In one experiment, milk was divided into three lots: Lot 1—control; Lot 2—heated at 66° C.—30 min. and cooled; and Lot 3—heated at 66° C.—30 min., cooled, and held for 24 hr. These samples were then heated to 90° C. for 30 min., accompanied by aspiration. The preheating temperature of 66° C. for 30 min. was selected because it was not sufficient to produce thiamine disulfide–reducing materials in milk.

A second series was conducted to determine the effect of combining low-temperature preheating and condensing on the subsequent production of active -SH groups by the heat treatment of the final product. Milk was preheated in 10-gal. milk cans at 63° C. for 30 min., and condensed to one-half its original volume in a stainless steel vacuum pan at from 46 to 49° C. The condensed product was then cooled and subsequently heated to 90° C. for 30 min. The control sample was obtained from the hot well after the preheating period. Results for both series are presented (Table 6).

The Series 1 data reveal that the low-temperature preheating decreased the H_2S about 37% and lowered the initial and the residual TDS values. Storage of the milk for 24 hr. following preheating has little additional effect. The preheated samples yielded relatively large quantities of H_2S without being accompanied by a corresponding reduction in the TDS values.

TABLE 6

Effect of low-temperature preheating and/or 1:2 concentration on the volatile and nonvolatile sulfhydryl content in heated milk

Sample	H_2S [a]	TDS values as cysteine-HCl	
		Initial [a]	Final [b]
	(γ/l)	*(mg/l)*	*(mg/l)*
SERIES I—preheated (av. 4 trials)			
Lot 1—Control, heated 90° C.—30 min.	428	20.4	16.2
Lot 2—Forewarmed: 66° C.—30 min. Heated 90° C.—30 min.	271	14.2	13.5
Lot 3—Forewarmed: 66° C.—30 min. Stored for 24 hr. Then heated 90° C.—30 min.	248	13.8	13.4
SERIES II—preheated and condensed [c] (av. 3 trials)			
Lot 1—Control, forewarmed 63° C.—30 min. Heated 90° C.—30 min.	232	12.3	9.6
Lot 2—Forewarmed: 63° C.—30 min./condensed 2:1 Heated 90° C.—30 min.	38	19.8	19.1

[a] Represents values obtained when the milk was heated to 90° C.
[b] After exhausting the H_2S.
[c] Milk was preheated and condensed in pilot-plant equipment.

Series 2 results also revealed that the preheating decreased both the volatile and the nonvolatile -SH concentrations created by subsequent heat treatment, in comparison to those expected from normal milk. The volatile -SH concentration in the condensed sample was extremely small. The TDS values for the condensed milk were approximately twice those in the uncondensed milk, indicating that no loss occurred during the condensing procedure.

Other observations revealed lyophilization of milk to have no appreciable effect on the quantity of heat-revealed -SH groups in the reconstituted product.

DISCUSSION

The simultaneous measurement of the H_2S and of the -SH groups responsible for reducing thiamine disulfide (TDS), as conducted in this study, allows an estimation to be made of the total -SH group reducing capacity in a heated milk system. Such a measurement also permits a reasonably accurate differentiation between the volatile and the nonvolatile portions of the reactive -SH groups. However, it must be recognized that the terms volatile and nonvolatile, as arbitrarily used in this study, do not necessarily infer a perfect separation of these two groups of -SH compounds, because of the limitations of the methods themselves. Furthermore, although the nonvolatile results (TDS values) are taken as an expression of -SH groups, it does not preclude the likelihood that other reducing compounds are involved.

In the application of the methods, and in order to differentiate as clearly as possible between the volatile and the nonvolatile compounds, three points were considered: first, the complete release of all the H_2S which is produced by the heat treatment; second, the maintenance of the product in a suitable condition, so that analysis for the nonvolatile (TDS values) portion could be made with accuracy; and third, the prevention of the formation of additional H_2S during

the process of removing that previously produced. The aspiration method adopted for the H_2S measurements met these desirable characteristics.

The aspiration method was more efficient than that used earlier by Townley and Gould *(15)*, and complete liberation of the H_2S was achieved.

Data obtained offer evidence that production of volatile -SH compounds is a two-step process: first, the production of materials which reduce TDS (excluding hydrogen sulfide); second, the decomposition of a portion of these to permit evolution of the hydrogen sulfide. Thus, momentary heating produced the greatest quantity of TDS-reducing materials at any given temperature above the critical temperature for -SH group production. This would suggest that if the exposure time to a given high temperature were sufficiently well controlled, a maximum of TDS-reducing materials might be produced without appreciable loss due to H_2S. Presumably, such treatment would result in a product with maximum antioxidant properties for the conditions involved.

At any given temperature and time within the critical range of reactive -SH group production, it appears that hydrogen sulfide is produced rather rapidly, and the production corresponds with a loss in the TDS values. However, a portion of the TDS-reducing materials is comparatively stable to decomposition and does not appear to be the precursor of the hydrogen sulfide. The question arises as to the conditions which will affect the proportion of stable and unstable TDS-reducing materials.

Once H_2S production begins, its major portion is created quickly. On the average, 84% of the total H_2S produced under the conditions of this study was removed from the sample during a 30-min. heating and aspirating period. This 363 γ/l of H_2S was equivalent to a loss of 3.7 mg. cysteine·HCl/l.

Removal of all the H_2S reduced the TDS values by approximately 20%. On a molar basis, the ratio of hydrogen sulfide released to the decrease in TDS values expressed as cysteine · HCl was approximately 1:2.

The finding that cream and buttermilk produce more H_2S than milk and whey is a corroboration of Townley and Gould's findings *(15)*. Buttermilk yielded about 38% more hydrogen sulfide than whole milk, 103% more than whey, and 11% more than cream. The initial TDS values correspond well with the quantity of the volatile -SH compounds obtained. The average molar ratio of the hydrogen sulfide released to the decrease in TDS values for whey, buttermilk, and cream were 1.0:2.7, 1.0:2.0, and 1.0:2.1, respectively. These results substantiate previous findings that the material associated with the fat is one of the principal sources of active -SH compounds.

The adverse effect of copper on heat-produced hydrogen sulfide in milk has been reported previously, but the effect of this metallic ion on the total -SH concentration is a new observation. This is of vital concern in the consideration of the antioxidant property of the heated product.

The results indicate that the volatile and nonvolatile -SH concentrations are affected adversely to an extent proportional to the concentration of the cupric ion. When 0.5 p.p.m. copper is added prior to or after heating, the molar ratio

of reaction between the hydrogen sulfide and the added copper was 1:1, exclusive of the change in the nonvolatile -SH compounds. In respect to changes in the quantity of hydrogen sulfide evolved, it made little difference whether the copper was added before or after heating. However, the decrease in TDS values was 10% greater when the copper was added after heating rather than before. This additional decrease possibly may be due to greater reactivity of the -SH groups (or other reducing groups) in the heated milk, which are measured by the TDS method.

Proper interpretation of the decrease of the TDS values, due to added copper and storage time, must include the possible loss of other milk constituents that contribute to the reduction of thiamine disulfide. Observations revealed that ascorbic acid reduced thiamine disulfide. Since ascorbic acid is oxidized quickly by copper, the total loss of TDS values on storage may be expected to reflect the loss of this constituent along with the -SH groups.

The observation that the addition of so-called stabilizing salts, such as calcium and phosphate, influences the quantity of reactive -SH groups produced by heat, points the way to additional studies involving the relationship of the salt and protein systems and -SH production of heated milk.

It is a common practice in the milk-condensing industry to heat milk prior to condensing, in order that heat stability may be increased and the viscosity in the finished product may be controlled. In this study, even low-temperature preheating, below the lowest temperature for active -SH production, caused a reduction of TDS values when the milk was subsequently heated. This indicates that a change is produced in the protein system by the forewarming treatment, which tends to protect it from subsequent alterations which give rise to the active -SH groups. Additional research is needed in this connection.

SUMMARY

A study was conducted in which the volatile and nonvolatile sulfhydryl (-SH) compounds of heated milk were measured simultaneously. This involved the determination of *(a)* the hydrogen sulfide (H_2S), by using aspiration with nitrogen to remove the gas from the milk, and *(b)* the reactive -SH groups by the thiamine disulfide (TDS) method. For the most part, samples were heated at 90° C. for 30 min., cooled to room temperature, and exhaustively aspirated. All of the H_2S was removed by the aspiration procedure used. TDS determinations were made when the temperature reached 90° C. and after each subsequent 30-min. aspirating period.

Momentary heating of the milk at 90° C. yielded the highest TDS values, which averaged (expressed as mg/l of cysteine · HCl) 21.0 for milk, 17.4 for whey, 22.8 for 35% cream, and 22.9 for buttermilk. H_2S liberation began essentially instantaneously after the production of the thiamine disulfide–reducing materials. Products heated to 90° C. for 30 min., cooled, and aspirated yielded the following amounts of H_2S expressed as gamma (γ) per liter: milk 433, cream 535, buttermilk 596, and whey 293.

The original TDS values for the products varied directly with the quantity of H_2S subsequently liberated, i.e., higher for ice cream and buttermilk than for whey. For all products, the decrease in TDS values resulting from liberation of H_2S was within the range of from 20 to 22%. The results obtained for milk reveal, in general, a decrease of 1.0 mg. in TDS value for each 0.1 mg. of hydrogen sulfide removed from the sample, or a molar ratio of 2:1.

The addition to the milk of 0.5 p.p.m. of copper before or after heating decreased the volatile and nonvolatile -SH content. When 0.5 p.p.m. copper was added, the quantity of hydrogen sulfide produced by heating was decreased by 64% in comparison to the control. The TDS values were lowered as the result of the copper-treatment of the milk as follows: 0.5 p.p.m. copper added before heating—17%; 0.5 p.p.m. copper added after heating—26%, and 2.0 p.p.m. copper added after heating—53%.

The addition of 0.025% calcium chloride or disodium phosphate to whole milk or whey before heating altered the amount of hydrogen sulfide and thiamine disulfide-reducing materials, when compared to the untreated milk, with the magnitude and direction of the effect being influenced by the type of salt and of milk system. These findings indicate the need for additional research on the relationship of the salt system of milk to reactive -SH production in heated milk.

Low-temperature preheat treatments markedly decreased the volatile and nonvolatile -SH content in milk or condensed milk subsequently heated to 90° C. for 30 min.

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BOVINE SEMEN METABOLISM. II. INFLUENCE OF SPERM CONCENTRATION AND INITIAL FRUCTOSE LEVEL ON FRUCTOLYTIC ACTIVITY[1]

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The influence of sperm concentration and initial levels of fructose on fructolysis were determined. It was found that sperm concentration and the initial fructose level of semen influenced the amount of the sugar used by spermatozoa, and a method for adjusting the fructolysis index for both factors was derived. Increasing the fructose level per ml. from 3.94 to 7.19 mg. in a 30-sample split-ejaculate study, increased its utilization from 2.00 to 3.23 mg. The fructose coefficient (Ku) was unaffected. This coefficient and adjusted fructose utilization may be useful in comparing semen quality and in detecting factors, other than sperm concentration and fructose level, which affect fructolysis. *Editor.*

The ability of bull spermatozoa to glycolyze fructose, glucose, and mannose to lactic acid was demonstrated by Redenz *(14, 15)*, and Killian *(8)* reported that human semen samples lost sugar and produced lactic acid during incubation. Comstock *(3)* reported that a positive correlation was found between sperm concentration and glycolysis in raw semen of rams, based upon differences in total reducing sugars. Mann *(9, 10, 11)* identified fructose as the reducing sugar of bull semen and proposed an "index of fructolysis" as an expression of metabolic activity.

A number of workers *(1, 2, 5, 7)* have demonstrated that there is a significant correlation between sperm concentration and various measures of fructolytic activity of bull semen, and it has been the practice to adjust fructose utilization values to the basis of 1 billion sperm by direct proportion in deriving a fructolysis index *(3, 5, 7, 12, 15)*.

The relationship between initial fructose levels and rate of fructolysis has not been well evaluated to date. Redenz *(15)* noted that the addition of glucose increased lactic acid production by washed bull sperm suspended in dialyzed serum. Salisbury *(18)* and Salisbury and VanDemark *(20)* reported that the addition of 58 to 116 mg. % glucose to bull semen in egg yolk–citrate buffer resulted in increased lactic acid production in samples incubated for one hour at 46.5° C. and in samples stored for ten days at 5° C. It has been demonstrated that bull spermatozoa utilize added glucose in preference to seminal fructose

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(22). Erb *et al. (5)* presented data graphically which showed the effect of initial seminal fluid fructose levels on amount of fructose utilized by bull sperm during one hour. In a study on isolated ciliary processes of beef eyes, de Roetth *(17)* concluded that an increase in the concentration of frucose or glucose in the perfusing medium, in the range of 0 to 200 mg. %, markedly increased the anaerobic lactic acid production.

Hopwood *et al. (7)* have used the decline in fructose level of semen with time, to express the rate of fructolytic activity as a first-order reaction. These authors reported that semen samples with high initial levels of fructose had lower slope values than samples with low initial levels of fructose, which indicated that this was not a true first-order reaction as expressed in terms of fructose levels.

Mixner *et al. (13)* presented the mathematical basis for the calculation of a fructolysis coefficient (K_u), which was defined as the fractional decline in sperm fructolytic activity per minute on an instantaneous basis. The K_u was calculated by the use of first-order rate principles, using the amounts of fructose utilized by bull spermatozoa in successive equal periods of incubation time. This measure was believed to be basically independent of sperm concentration, initial level of fructose in semen, and time from ejaculation. The mean fructolysis coefficient for 70 semen samples was 0.0121, with a mean biological half-life of 57.4 min.

The purpose of this study was to determine the influence of sperm concentration and initial levels of fructose on certain measures of fructolysis and, if appropriate, to derive a method for adjusting the fructolysis index for the influence of both factors. A brief report has been made *(6)*.

MATERIALS AND METHODS

The semen samples used in these studies were collected by the use of an artificial vagina, from Holstein and Guernsey bulls located in a stud at the Dairy Research Farm of the New Jersey Agricultural Experiment Station. Semen samples were collected from the bulls weekly. Only those semen samples were used which met the quality standards for routine artificial insemination. The time from sample collection to the start of incubation averaged about five minutes and in no case was more than ten minutes.

Immediately after collection, 0.5 ml. of semen was added to 2.0 ml. of 2.9% sodium citrate dihydrate buffer at 37° C. in stoppered incubation tubes and gently mixed. Samples were prepared for the determination of initial fructose level by immediately adding 0.5 ml. of the buffered semen to 3.5 ml. of 10% trichloroacetic acid in heavy-duty centrifuge tubes. These tubes were centrifuged at 2,000 Relative Centrifugal Force for ten minutes and then 2.0 ml. of the supernatant fluid was pipetted into 8.0 ml. of 10% trichloroacetic acid and mixed. These steps were repeated after 20, 40, and 60 min. of incubation at 37° C.

Fructose concentration was determined photometrically on a 2.0-ml. sample of the diluted supernatant, using the method of Roe *(16)* as modified by Erb

et al. (4, 5). Triplicate standard fructose tubes were run with each series. A Klett-Summerson photometer was used for the fructose measurements (Filter No. 54) and for the turbidimetric determination of sperm concentration (Filter No. 66), after the method of Salisbury *et al. (19)*.

The fructolysis coefficients (K_u) were calculated by the method of Mixner *et al. (13)*, using the first two 20-min. incubation periods.

Statistical analyses of the data followed the methods outlined by Snedecor *(21)*.

RESULTS AND DISCUSSION

Variance associated with measures of fructolysis. When the variations in buffers, dilution rates, incubation times and temperatures, and assay modifications used by different investigators were considered, the need for an evaluation of the variation associated with the specific techniques used in this study was apparent. Accordingly, duplicate tubes were incubated for each of ten semen ejaculates, and duplicate fructose assays were made from each of the duplicate incubation tubes after 0, 20, 40, and 60 min.

Mean sperm concentration for these samples was 1.156 billion and mean initial fretose level was 4.74 mg. per ml. Mean fructose utilizations were 1.01, 1.50, and 2.00 mg. per ml. of semen for the 20-, 40-, and 60-min. incubation periods, respectively.

An analysis of variance of the data is presented (Table 1) and the expectations of mean squares and estimated variances (Table 2). The expectations of

TABLE 1

Analysis of variance of measures of fructolysis

Source of variation		Degrees of freedom	Mean square			
			Fructose utilized in:			
	(symbol)		20 min.	40 min.	60 min.	K_u
Ejaculates	E	9	1.4627*	1.6801*	2.4695*	0.00665
Tubes in ejaculates	T(E)	10	0.2734*	0.2818*	0.3140*	0.00278
Assays in tubes in ejaculates	A(T)(E)	20	0.0698	0.0519	0.0411	0.00226
Total		39				

* Significant at 1% level ($P < 0.01$).

mean squares (Table 2) showed that the mean square for T(E) was the appropriate error term for testing the significance of differences among ejaculates. Thus, the use of replicate incubation tubes is needed to supply an error term for testing differences among ejaculates. With respect to fructose utilizations in 20, 40, and 60 min., an increasing percentage of total variance is associated with ejaculates (E) in the longer incubation times, reflecting the added accuracy of a longer incubation period. An examination of the error variances associated with the fructolysis coefficients (K_u) suggests that 20-min. incubation periods are too short to be used as a basis of this calculation, since only 27.7% of total variance was associated with ejaculates (E), the balance being error variance. Therefore, any findings or indications in this paper of whatever nature associ-

TABLE 2

Expectation of mean squares and estimated variances for measures of fructolysis

Source of variation	Expectation of mean squares		Variance and variance percentages			
			Fructose utilized in:			
		Variance	20 min.	40 min.	60 min.	K_u
E	$\sigma^2_{A(T)(E)} + 2\sigma^2_{T(E)} + 4\sigma^2_E$	V	0.2973	0.3496	0.5866	0.000968
		%V	63.4	67.7	76.8	27.7
T(E)	$\sigma^2_{A\,T(E)} + 2\sigma^2_{T(E)}$	V	0.1018	0.1159	0.1364	0.000260
		%V	21.7	22.3	17.8	7.5
A(T)(E)	$\sigma^2_{A\,T(E)}$	V	0.0698	0.0519	0.0411	0.002261
		%V	14.9	10.0	5.4	64.8
		V	0.4689	0.5165	0.7641	0.003489
Total		%V	100.0	100.0	100.0	100.0

ated with the fructolysis coefficient *(K_u)* must be accepted with reservation. Presumably, the error variance could be decreased by increasing the lengths of the incubation periods.

Relation of sperm concentration and initial fructose level to various measures of fructolysis. Seventy semen ejaculates from 12 bulls were used in a study to determine the interrelationships among initial fructose level, sperm concentration, and various measures of fructolysis. Mean sperm concentration was 1.220 billion and mean initial fructose level was 4.96 mg. per ml. of semen. Mean fructose utilizations were 0.88 mg. per ml. in 20 min., 1.57 mg. per ml. in 40 min., and 2.12 mg. per ml. in 60 min. The mean fructolysis coefficient *(K_u)* was 0.0121, giving a mean biological half-life of 57.4 min.

Certain zero-order, partial, and multiple correlations calculated from the data on the various semen measures on "total," "among-bull," and "ejaculates-within-bull" bases are presented (Table 3).

These data showed that higher sperm concentrations and initial fructose levels of semen were associated with higher fructose utilizations during 20, 40, and 60 min. of incubation; whereas, at the same time, these factors had no effect on the fructolysis coefficient K_u. The multiple correlation, 0.63, of 60-min. utilization with sperm concentration and initial fructose level on an ejaculate-in-bull basis, showed the combined influence of sperm concentration and initial fructose level on fructose utilization in one hour at 37° C. These data confirm the reports of others *(1, 2, 3, 5, 7)* with regard to the influence of sperm concentration on fructose utilization, but also indicate the important influence of initial fructose level on fructose utilization *(5, 15, 17, 19, 20)*.

The mean biological half-life of 57.4 min. determined from these data, as a measure of the rate of decline in fructolysis, indicates that it might be appropriate to base the calculation of the fructolysis coefficient *(K_u)* on two one-hour incubation periods, instead of the two 20-min. periods, as was done in this study. This should greatly increase the accuracy of the K_u measure and the validity of correlations associated with this measure.

In a further analysis of this study, fructose utilization values were adjusted for differences in sperm concentration and initial fructose levels by multiple

TABLE 3

Correlations among various semen characteristics

Correlated factors	Coefficients of correlation and degrees of freedom		
	Total	Among bulls	Ejaculates within bulls
Zero-order correlations — degrees of freedom	68	10	57
Sperm concentration *versus*			
initial fructose level	−0.02	−0.34	0.06
20-min. utilization	0.20	0.36	0.18
40-min. utilization	0.35[b]	0.54	0.31[a]
60-min. utilization	0.30[a]	0.44	0.27[a]
K_u	0.00	0.07	−0.02
Initial fructose level *versus*			
20-min. utilization	0.40[b]	0.37	0.41[b]
40-min. utilization	0.45[b]	0.30	0.48[b]
60-min. utilization	0.56[b]	0.43	0.59[b]
K_u	−0.04	0.17	−0.10
K_u *versus*			
20-min. utilization	0.09	0.26	0.06
40-min. utilization	0.11	0.22	0.08
60-min. utilization	0.14	0.21	0.12
Partial correlations — degrees of freedom	67	9	56
Independent of initial fructose level, sperm concentration *versus*			
20-min. utilization	0.23	0.56	0.17
40-min. utilization	0.40[b]	0.71[a]	0.32[a]
60-min. utilization	0.38[b]	0.69[a]	0.30[a]
Independent of sperm concentration, initial fructose levels *versus*			
20-min. utilization	0.42[b]	0.57	0.41[b]
40-min. utilization	0.49[b]	0.61[a]	0.49[b]
60-min. utilization	0.60[b]	0.69[a]	0.59[b]
Multiple correlations — degrees of freedom	67	9	56
Sperm concentration and initial fructose level with			
20-min. utilization	0.45[b]	0.64	0.44[b]
40-min. utilization	0.57[b]	0.74[a]	0.56[b]
60-min. utilization	0.64[b]	0.76[a]	0.63[b]

[a] Significant at 5% level ($P < 0.05$).
[b] Significant at 1% level ($P < 0.01$).

covariance, and showed no significant differences among bulls either before or after adjustment, after 20-, 40-, or 60-min. incubation. There was no appropriate error term for testing differences among ejaculates-within-bull in fructose utilizations.

The effect of added fructose on fructolysis. Thirty semen samples (three samples from each of ten bulls) were utilized in a split-ejaculate study, to determine more directly the effect of initial fructose level on fructose utilization. Fructose was added to the sodium citrate buffer used for one-half of each ejaculate, so that there was a mean increase of approximately 80% in the initial fructose level of the diluted semen in the fructose tubes over that in the control tubes. Duplicate tubes were incubated for both the control and the fructose part of each ejaculate. Fructose utilizations were determined after 20, 40, and

TABLE 4

Effect of added fructose on measures of fructolysis

Incubation tubes	Initial level of fructose	Fructose utilized in: 20 min.	40 min.	60 min.	K_u
		(mg/ml)			
Control	3.94	0.74	1.51	2.00	0.0111
Added fructose	7.19	1.28	2.33	3.23	0.0087

60 min. of incubation and the fructolysis coefficients (K_u) were also determined (Tables 4 and 5).

Fructose addition markedly stimulated the utilization of fructose by sperm at all incubation times, and at the same time it did not significantly influence the fructolysis coefficients (K_u). These findings confirm the correlation studies already presented, and serve to emphasize that fructose utilization (but not the fructolysis coefficient) is markedly influenced by the level of fructose in the medium. Both studies thus tend to confirm the proposal of Mixner *et al. (13)*, that the measurement of fructolytic activity of bovine semen may best be expressed as the fructolysis coefficient, which is now demonstrated to be independent of both sperm concentration and initial fructose levels.

Adjustment of fructose utilization for effects of sperm concentration and initial fructose level. The data which served as the basis for the correlation study were also used for adjusting fructose utilization (Y) for the effects of sperm concentration in billions per ml. (X_1), and initial fructose level in mg. per ml. (X_2). For this purpose, a multiple regression equation for adjustment purposes was adapted:

$$\text{adjusted } Y = Y - b_1 (X_1 - X'_1) - b_2 (X_2 - X'_2)$$

where Y is the actual amount of fructose utilized; b_1 is the partial regression of fructose utilization on sperm concentration independent of initial fructose level; b_2 is the partial regression of fructose utilization on initial fructose level independent of sperm concentration; X'_1 is a convenient arbitary value for

TABLE 5

Analysis of variance for split-ejaculate study of added fructose

Source of variation	*(symbol)*	Degrees of freedom	Mean square: Fructose utilized in: 20 min.	40 min.	60 min.	K_u
Among bulls	B	9	1.251	3.062	6.386	0.00612
Ejaculates in bulls	E(B)	20	0.922	2.730[a]	2.892[b]	0.00643
Fructose level	F	1	8.876[b]	19.992[b]	44.835[b]	0.00018
F × B	FB	9	0.519	1.822	3.660[b]	0.00616
F × E(B)	FE(B)	20	0.614[b]	1.151[b]	0.948[b]	0.00771[b]
Tubes in ejaculates in bulls	T(E)(B)	60	0.168	0.174	0.201	0.00276
Total		119				

[a] Significant at 5% level ($P < 0.05$).
[b] Significant at 1% level ($P < 0.01$).

sperm concentration (1.0 billion per ml.); and X'_2 is a convenient arbitrary value for initial fructose level (5.0 mg. per ml. semen). The two arbitrary values are near the observed means. These partial regression coefficients must be calculated for any given set of experimental conditions. The adjustment equations for this study, calculated on ejaculates-in-bull basis, were

(1) 20-min., adjusted $Y = Y - 0.2524\ (X_1 - 1.000) - 0.1424\ (X_2 - 5.00)$

(2) 40-min., adjusted $Y = Y - 0.5446\ (X_1 - 1.000) - 0.1996\ (X_2 - 5.00)$

(3) 60-min., adjusted $Y = Y - 0.6789\ (X_1 - 1.000) - 0.3544\ (X_2 - 5.00)$.

The adjusted mean fructose utilizations per ml. for the 70 semen samples used in the correlation study were 0.84 mg. in 20 min., 1.50 mg. in 40 min., and 1.99 mg. in 60 min. The application of the adjustment equations may be illustrated by utilizing the data in the fructose addition study (Table 4). When the data for the 60-min. fructose utilizations were substituted in Equation 3, the control utilization was adjusted to 2.23 mg. and the utilization for added fructose was adjusted to 2.30 mg. Similarly, fructose utilization of individual semen samples may be adjusted to the common basis of one billion sperm and 5.0 mg. per ml. of initial fructose. A "fructolysis index" so calculated should be much superior to the usual fructolysis index, in which utilizations are corrected on a simple proportionality basis for the effects of sperm numbers only.

Since both sperm concentration and initial fructose level significantly influence the utilization of fructose by sperm, any measure of fructolysis which is to be useful in comparing the quality of semen samples, or in detecting the influence of other factors on fructolysis, must be independent of these two factors. Two new approaches to this problem are now available, as the result of this and a previous study *(13)*. An adjustment equation using partial regression coefficients may be used to correct fructose utilizations to the common basis of one billion sperm and 5.0 mg. per ml. of initial fructose. A second method *(13)* utilizes first-order rate principles to calculate a slope value or fructolysis coefficient (K_u), which the present study has shown to be independent of the effects of sperm numbers and initial fructose levels. Additional information needs to be accumulated, to determine to what extent the adjusted fructolysis index and the fructolysis coefficient measures are correlated.

SUMMARY

A multiple regression study of 70 semen samples showed that both the sperm concentration and initial fructose level of semen samples influenced the amount of fructose utilized by bovine spermatozoa. A highly significant multiple correlation ($R = 0.63$) was found on an ejaculate-in-bull basis between them and fructose utilization in 60 min.

The addition of fructose to semen in a 30-sample split-ejaculate study, so that the mean initial fructose level was increased from 3.94 to 7.19 mg. per ml., resulted in an increase in 60-min. utilization from 2.00 to 3.23 mg.

In neither study did the sperm concentration or the initial fructose level have any effect on the fructolysis coefficient (K_u), which is a measure of rate of fructolysis based on first-order rate principles.

Adjustment by regression formula for the influence of both sperm concentration and initial fructose level on fructose utilization was proposed. Application of this formula to 60-min. utilization data in the fructose addition study resulted in the adjustment of the fructose and control means to equal fructose utilizations.

It is suggested that the fructolysis coefficient and the adjusted fructose utilization are two measures of fructolysis which may be useful in the comparison of semen sample quality, and in the detection of factors, other than sperm concentration and fructose level, which influence fructolysis.

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EFFECTS OF D-LIMONENE AND α-D-PINENE ON IN VITRO CARBOHYDRATE DISSIMILATION AND METHANE FORMATION BY RUMEN BACTERIA

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The effects of D-limonene and α-D-pinene on the in vitro dissimilation of carbohydrates and methane formation by rumen bacteria were studied by enrichment culture techniques. 9×10^{-4} *M* D-limonene, but not 9×10^{-4} *M* α-D-pinene, was toxic to stabilized rumen cultures which produced methane from acetic acid. Methane production from formic acid by rumen cultures was not affected by these terpenes. Cellulose digestion, but not starch and cellobiose dissimilation by rumen bacteria, was inhibited by higher concentrations of both terpenes. *Editor.*

In attempts to apply the anaerobic methane fermentation to citrus waste–water as a method of sewage disposal, McNary *et al. (6)* observed that the fermentation was inhibited by D-limonene from citrus peel oil. The data indicate that the terpene was toxic to the methane bacteria, and that conversion of carbohydrate to methane and carbon dioxide by the mixed flora was inhibited at the stage of volatile acid utilization. Buswell and Mueller *(3)* later reported that D-limonene inhibited gas production by domestic sewage cultures stabilized to acetic acid.

Since analogous fermentations may occur in the rumen, experiments were designed to determine the effects of D-limonene and a related terpene, α-D-pinene, on: *(a)* methane production by rumen-enrichment cultures stabilized to acetic or formic acid, and *(b)* the in vitro degradation of cellulose, starch, and cellobiose by mixed rumen microorganisms. The results of these experiments are reported herein.

METHODS AND MATERIALS

Bacteriological. The fermentation apparatus used in this study was essentially that described by Buswell *et al. (4)*. Enrichment cultures of methane bacteria from bovine rumen fluid were established and maintained in the manner reported by Oppermann *et al. (8)*. Depending upon the degree of culture activity, 0.9 to 3.6 m*M* of formic acid, or 0.65 to 2.6 m*M* of acetic acid, was added daily to the stabilized cultures.

In carbohydrate dissimilation tests, the fermentation flasks contained 1.5 g. substrate, 40 ml. mineral salt solution *(2)*, 85 ml. tap water, and 125 ml. freshly drawn bovine rumen fluid, which had been filtered through cheese cloth. Finely ground cellulose, corn starch, and cellobiose were used as substrates. After the apparatus was assembled and loaded, the fermentation mixture was flushed with a stream of O_2-free CO_2, sealed, and incubated at 38° C. on an electromagnetic

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stirrer to provide gentle agitation. Cellulose fermentations were incubated for 48 hr. Starch and cellobiose fermentations were incubated 18 hr. The data from these trials have been corrected for endogenous values obtained from fermenters to which substrate was not added.

Concentrations of from 9×10^{-4} to 4.5×10^{-3} *M* D-limonene or α-D-pinene were obtained in the fermentation mixtures by calculated additions of a 10% ethanolic solution of the terpene. Control fermentations were treated with equivalent levels of ethanol.

Chemical. The gases produced during fermentation were collected by the displacement of acidified (pH 1.0) saturated aqueous Na_2SO_4, and the volume recorded after adjustment to atmospheric pressure. Gas composition was determined by absorption-combustion procedures, employing a Model No. 35-802 Burrell apparatus.

The concentrations of the individual acids in the fermentation mixture were measured by standard chromatographic procedure. Six grams of Celite 535 was acidified with 2.4 ml. of 0.2 *N* H_2SO_4, slurried in acid-equilibrated hexane, and packed in a 300×12 mm. chromatograph tube. One ml. of fermentation fluid at pH 1.5 was mixed with 2 g. Celite and packed on the top of a prepared column. The chromatogram was developed and the acids removed by elution with butanol-hexane solvents *(7)*. The eluate was collected in 5-ml. fractions, and titrated to a bromthymol blue end-point with 0.01 *N* alcoholic KOH in a stream of CO_2-free air. It should be noted that lactic and succinic acid are eluted together by the solvent system employed. This fraction is designated as lactic acid in this paper.

Carbohydrates were determined colorimetrically by the anthrone method of Viles and Silberman *(9)*, and were read at 660 mμ in an Evelyn photoelectric colorimeter. The supernatant of centrifuged fermentation aliquots was used in soluble carbohydrate determinations. The washed sediment from similarly centrifuged aliquots was employed in cellulose measurements. A standard curve prepared from the specific carbohydrate being determined was run with each series of samples analyzed.

RESULTS

The data summarized in Table 1 show the effects of D-limonene on the activity of enrichment cultures stabilized to acetic acid. Cultures to which 9×10^{-4} *M* D-limonene was added, utilized an average of 0.72 meq. of acetic acid during the test period, as compared to the utilization of 2.75 and 3.42 meq. of acetic acid by the 4.5×10^{-4} *M* D-limonene treated and control cultures, respectively. The volume of gas produced was proportional to the amount of acetic acid metabolized, and the addition of D-limonene did not alter the composition of the gas.

In similar experiments it was noted that cultures given a single dosage of 9×10^{-4} *M* D-limonene continued to decrease in activity until they were almost completely inactive, usually within five days after treatment. Cultures treated with 4.5 *M* D-limonene partially overcame the inhibition, and produced gas at a relatively constant but reduced volume as compared to the controls. Data

TABLE 1

Effect of D-*limonene on acetate utilization and methane production by stabilized rumen cultures*[a]

Treatment	Acetic Acid			Gas production		
	0 time (*meq.*)	30 hr. (*meq.*)	Utilized (*meq.*)	Theoretical (*cc.*)	Actual (*cc.*)	Composition (CO_2: CH_4)
None	3.90	0.48	3.42	164	149	0.98
4.5×10^{-4} *M* D-Limonene	4.78	2.03	2.75	132	128	0.96
9×10^{-4} *M* D-Limonene	4.10	3.38	0.72	36	43	1.06

[a] Data represent mean values obtained from three cultures in each treatment group.

obtained from Fermenters No. 3, 4, and 11 (as shown in Table 2) illustrate the activity of cultures five days after treatment with 9×10^{-4} *M* D-limonene. Table 2 also shows the effects of a second addition of inhibitor five days after the first addition. The response of Fermenter No. 2 to the second 4.5×10^{-4} *M* D-limonene treatment indicates that the effect of the two treatments was not additive, and that a portion of the terpene added originally was metabolized, or otherwise inactivated, by the time the second addition was made.

The effects of D-limonene on gas production by formic acid–stabilized cultures are illustrated in Figure 1. The presence of 4.5×10^{-4} *M* D-limonene in the fermentation medium did not appreciably affect gas production; whereas, the 9×10^{-4} *M* concentration reduced the rate of gas formation. However, unlike the acetate cultures, the formate cultures returned to pretreatment levels of activity on the day after treatment. Similarly, gas production by acetate cultures and by formate cultures was only slightly retarded by 4.5×10^{-4} and 9×10^{-4} *M* α-D-pinene. In both instances, the treated cultures produced gas at normal rates when substrate was added the day following treatment.

Preliminary experiments suggested that the in vitro digestion of cellulose by the flora of fresh rumen fluid was markedly inhibited by 9×10^{-3} *M* D-limonene,

TABLE 2

Effect of a second addition of D-*limonene on gas production by stabilized rumen cultures*[a]

Fermenter	Treatment	Previous treatment	Total gas production (*cc.*)		
			(*3 hr.*)	(*5 hr.*)	(*7 hr.*)
3	None	None	60	90	118
4	None	4.5×10^{-4}	50	70	92
11	None	9.0×10^{-4}	5	5	6
1	4.5×10^{-4} *M* L	None	41	61	79
2	4.5×10^{-4} *M* L	4.5×10^{-4}	28	90	52
6	4.5×10^{-4} *M* L	9.0×10^{-4}	8	8	8
7	9.0×10^{-4} *M* L	None	15	20	21
8	9.0×10^{-4} *M* L	4.5×10^{-4}	10	11	11
12	9.0×10^{-4} *M* L	9.0×10^{-4}	11	12	12

[a] Cultures same as in Table 1. Second addition of D-limonene made as indicated, five days following first addition. Daily substrate added between treatments, in accordance with culture activity.

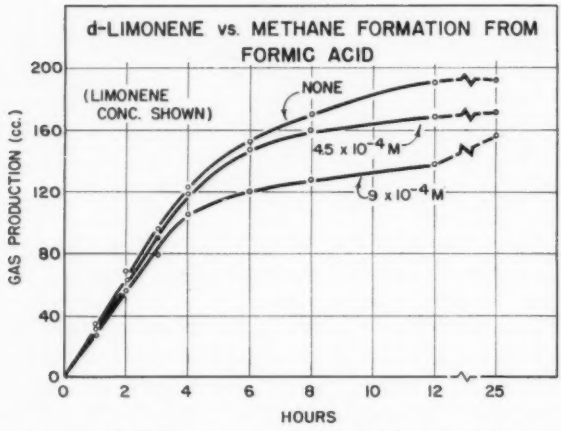

FIG. 1. *Conditions:* Methanogenic rumen cultures (three each treatment) stabilized to formic acid were treated with D-limonene at zero time, as indicated after addition of 3.6 m*M* of substrate.

but only slightly inhibited by 9×10^{-4} *M* D-limonene. The in vitro digestion of starch or cellobiose was inhibited by 9×10^{-3} D-limonene only in the initial stages of incubation, after which the inhibition was overcome and the carbohydrate digestion equalled that of the controls. Starch and cellobiose digestion by rumen fluid was not inhibited by 9×10^{-4} D-limonene.

In consideration of these preliminary results, a series of experiments was conducted to determine the effects of 9×10^{-4} *M*, 2.3×10^{-3} *M*, and 4.5×10^{-3} *M* concentrations of D-limonene and α-D-pinene on cellulose, starch, and cellobiose dissimilation by samples of fresh, whole rumen fluid from the same animal. As shown (Table 3), the inhibition of cellulose digestion by rumen bacteria became more pronounced as the terpene concentration was increased; α-D-pinene inhibited cellulose digestion to a greater extent than did the corresponding levels of D-limonene. Little, if any, inhibition of the digestion of starch and cellobiose was caused by either D-limonene or α-D-pinene, except in the starch fermentation containing the greatest concentration of α-D-pinene. In this instance, the final starch hydrolysis was somewhat lower than the control.

Data relative to the amounts and composition of the gas and acidity produced by the just-mentioned fermentations are summarized in Tables 4 and 4a. In general, the total net gas volume and net increase in volatile plus lactic acidity was directly related to the amount of cellulose digested. The data show that the addition of either D-limonene or α-D-pinene caused a slight increase in the molar concentrations of acetic and butyric acids, and a marked decrease in the molar percentage of propionic acid resulting from cellulose digestion. The magnitude of the changes in the concentration of the fermentation acids was not directly related to the concentration of the terpenes used. The concentration of methane in the gas produced during the cellulose fermentation was increased, and the

TABLE 3

Effect of D-*limonene and* α-D-*pinene on carbohydrate digestion by mixed rumen flora*

	Per cent carbohydrate digestion									
	Cellulose[a]				Starch[a]				Cellobiose[a]	
Additions	*(6 hr.)*	*(15 hr.)*	*(24 hr.)*	*(48 hr.)*	*(3 hr.)*	*(6 hr.)*	*(18 hr.)*	*(24 hr.)*	*(6 hr.)*	*(16 hr.)*
None	14	48	74	92	9	22	93	97	58	96
9×10^{-4} limonene	10	27	44	84	9	18	82	87	60	84
2.3×10^{-3} limonene	11	17	22	69	5	11	94	95	82	97
4.5×10^{-3} limonene	9	19	20	34	7	12	94	93	89	97
None	12	36	44	83	3	13	92	94	98	99
9×10^{-4} pinene	0	1	2	48	10	22	94	98	69	99
2.3×10^{-3} pinene	0	1	2	10	5	13	96	98	83	99
4.5×10^{-3} pinene	0	1	2	7	12	26	65	72	78	99

[a] 1.5 g. substrate per 250 cc. T.V. fermentation fluid.

carbon dioxide decreased, in those fermenters treated with the two higher levels of D-limonene. Analysis of the small volume of gas collected from the fermentations treated with the higher levels of α-D-pinene was not feasible.

The addition of D-limonene to starch fermentations caused an increase in the molar concentration of butyric acid and a decrease in the molar percentage of acetic acids. These changes were more pronounced as the concentration of D-limonene was increased. The molar concentration of propionic acid was not greatly affected by limonene. In contrast to the controls, lactic acid was found in the fermentations treated with the higher levels of D-limonene. The accumulation of lactic acid was more pronounced in starch fermentations treated with α-D-pinene. Increased levels of α-D-pinene also caused a decrease in the concentration of propionic acid. The gas collected from the control starch fermentation contained more methane than did the gas from the fermentations treated with 4.5×10^{-3} *M* terpene.

The relative concentration of butyric acid from cellobiose fermentation was not greatly affected by the terpenes. The proportion of propionic acid was unaffected by pinene, but was decreased as the concentration of limonene was increased. Both terpenes decreased the molar percentage of acetic acid and increased the concentration of lactic acid in the cellobiose fermentation fluid.

DISCUSSION

The foregoing data have shown that D-limonene is toxic to rumen bacteria capable of converting acetic acid to methane and carbon dioxide. In this regard, these rumen cultures resemble acetate utilizing methane cultures of sewage origin *(3)*.

The relative insensitivity of formate cultures suggests that D-limonene in low concentrations inhibits the acetate cleavage reactions which yield methane *(5)*,

TABLE 4

Effect of D-*limonene on carbohydrate digestion and acid and gas production by mixed rumen flora*

Substrate	D-Limonene conc. (*M*)	Substrate digestion (%)	Metabolic gas			Fermentation acids				
			Vol. (cc.)	CO_2 (%)	CH_4 (%)	Total (*mM*)	Butyric (*M*%)	Propionic (*M*%)	Acetic (*M*%)	Lactic (*M*%)
Cellulose[a]	None	74	320	82	12	11.7	12.7	28.2	59.1	0.0
	9×10^{-4}	44	190	85	11	7.5	15.2	17.4	65.0	2.4
	2.3×10^{-3}	22	70	75	20	3.1	15.4	15.9	68.7	0.0
	4.5×10^{-3}	20	60	65	26	2.8	19.0	15.1	65.9	0.0
Starch[b]	None	93	450	75	21	10.7	15.1	21.4	63.5	0.0
	9×10^{-4}	82	425	78	16	11.4	18.7	20.8	60.5	0.0
	2.3×10^{-3}	94	365	77	15.8	12.3	23.6	12.7	53.0	10.7
	4.5×10^{-3}	94	355	76	16.0	15.0	24.3	22.3	48.4	5.0
Cellobiose[c]	None	58	255	82.4	15.0	7.5	15.7	22.0	62.2	0.0
	9×10^{-4}	60	215	82.0	14.2	8.0	13.8	17.4	64.4	4.6
	2.3×10^{-3}	82	310	80.2	17.8	11.2	14.9	13.0	51.4	20.7
	4.5×10^{-3}	89	255	86.4	8.4	13.7	10.7	9.9	38.0	41.7

[a] 24-hr. incubation as shown in Table 3.
[b] 18-hr. incubation as shown in Table 3.
[c] 6-hr. incubation as shown in Table 3.

TABLE 4a

Effect of α-D-*pinene on carbohydrate digestion and acid and gas production by mixed rumen flora*

Substrate	α-D-Pinene conc. (*M*)	Substrate digestion (%)	Metabolic gas			Fermentation acids				
			Vol. (cc.)	CO_2 (%)	CH_4 (%)	Total (*mM*)	Butyric (*M*%)	Propionic (*M*%)	Acetic (*M*%)	Lactic (*M*%)
Cellulose[a]	None	83	510	80	17	9.9	12.3	28.6	59.7	0.0
	9×10^{-4}	48	315	84	15	7.3	17.4	14.3	68.3	0.0
	2.3×10^{-3}	10	10	.....	.....	1.4	18.8	16.0	65.2	0.0
	4.5×10^{-3}	7	10	.....	.....	1.0	16.0	16.5	67.5	0.0
Starch[b]	None	92	385	76.8	15.8	11.9	19.0	27.0	54.0	0.0
	9×10^{-4}	94	370	74.4	14.6	13.3	20.0	12.9	49.0	16.8
	2.3×10^{-3}	96	300	76.2	13.0	11.6	14.8	19.0	50.2	16.0
	4.5×10^{-3}	65	140	78.8	9.0	12.3	15.3	11.9	58.8	14.2
Cellobiose[c]	None	98	275	78.8	16.0	12.3	12.9	22.0	65.1	0.0
	9×10^{-4}	69	195	82.0	15.2	11.7	14.6	21.0	64.3	0.0
	2.3×10^{-3}	83	250	79.4	15.4	20.2	18.0	20.6	58.1	3.3
	4.5×10^{-3}	78	190	85.2	11.4	15.6	17.1	20.1	27.0	35.8

[a] 24-hr. incubation as shown in Table 3.
[b] 18-hr. incubation as shown in Table 3.
[c] 6-hr. incubation as shown in Table 3.

and that methanogenesis via carbon dioxide reduction *(1)* is not appreciably affected by this compound. α-D-Pinene in concentrations equivalent to the toxic levels of D-limonene did not inhibit the formation of methane from either acetic or formic acid by rumen cultures. Thus, the methane inhibitory effect exhibited by D-limonene is not common to all terpenes.

The 9×10^{-4} concentration of D-limonene which markedly inhibited the conversion of acetate to methane in stabilized cultures permitted the digestion of appreciable quantities of cellulose by rumen microorganisms. As the concentration of D-limonene or α-D-pinene was increased, the digestion of cellulose, gas formation, and acid production decreased proportionally. On the basis of these criteria, the cellulolytic bacteria in rumen fluid appear to be more sensitive to α-D-pinene than to D-limonene.

The degree of digestion of starch and cellobiose by rumen bacteria was not affected by either D-limonene or α-D-pinene. However, these terpenes appear to have partially altered the distribution of the fermentation end-products. The accumulation of considerable quantities of lactic acid in the presence of the higher concentrations of D-limonene and α-D-pinene suggests that the metabolism of lactate by the mixed rumen flora was partially blocked by the terpenes.

SUMMARY

9×10^{-4} *M* D-limonene, but not 9×10^{-4} *M* α-D-pinene, was toxic to stabilized rumen cultures which produce methane from acetic acid. Methane production from formic acid by rumen cultures was not affected by these terpenes.

Cellulose digestion, but not starch and cellobiose dissimilation by rumen bacteria, was markedly inhibited by higher concentrations of D-limonene or α-D-pinene.

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c 6-hr. incubation as shown in Table 3.

LIVABILITY OF SPERMATOZOA IN DILUTERS WITH CITRATE AND BICARBONATE BUFFERS AND YOLK

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The objectives of this paper were to compare yolk-glucose-bicarbonate with yolk-citrate, and to study the possible reasons for differences observed by the authors and by reports in the literature. The livability of spermatozoa in concentrations of 15×10^6/ml in yolk-glucose-bicarbonate was inferior to that in yolk-citrate, but there was no difference with sulfanilamide or when the concentration was 180×10^6/ml. At the lower concentration the pH of yolk-glucose-bicarbonate remained at about 7.00, but with 180×10^6/ml. the pH was 6.81 after 10 days. It was postulated that metabolism of spermatozoa at the lower concentration in yolk-glucose-bicarbonate was stimulated by the higher pH, but that sulfanilamide prevents this stimulation. *Editor.*

Much research has been done by various workers on diluters for bovine spermatozoa. Among these are Kampschmidt *et al. (2, 3)*, who reported that electrolytes were detrimental to bull spermatozoa during storage at 4 and 7° C. As a consequence, they developed a diluter consisting of egg yolk, glucose, and sodium bicarbonate which supported spermatozoa more satisfactorily than yolk-citrate at dilution levels of 1 : 9 or 1 : 10.

This paper reports results of experiments which demonstrate, *(a)* the inferiority of yolk-glucose-bicarbonate when compared with yolk-citrate containing 15×10^6 spermatozoa per milliliter, *(b)* the possible reasons for the differences between these results and those reported by Kampschmidt *et al.*, and *(c)* the ability of sulfanilamide to eliminate these differences.

METHODS AND PROCEDURES

Experiment I. Livability in yolk-citrate and yolk-glucose-bicarbonate. The data in this experiment are from a study comparing directly a number of different diluters. Only the comparison of yolk-citrate and yolk-glucose-bicarbonate diluters will be reported here.

Yolk-glucose-bicarbonate (YGB) consisted of one part yolk, four parts 5% glucose solution, and one part of a 1.3% sodium bicarbonate solution *(2, 3)*. The yolk-citrate diluter included one part of yolk and one part of a 2.9% solution of sodium citrate dihydrate. Dihydrostreptomycin sulfate was added to both diluters at a concentration of 500 γ per milliliter. In each, semen was diluted to have 15×10^6 spermatozoa per milliliter. Motility observations were made after storage at 5° C. for two, four, and ten days. After each interval of storage, motility estimations were made on each sample independently by two workers, and the two values were averaged.

Progressive motilities of 17 samples of bull spermatozoa after storage in YGB for two, four, and ten days averaged 53, 31, and 3%, and in YC, 53, 46, and 34%.

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Experiment II. Concentration of spermatozoa. This study was carried out to determine if the unsatisfactory results with the yolk-glucose-bicarbonate diluter in the preceding experiment could be explained by the low concentration of spermatozoa.

The general procedures were similar to those in Experiment I. pH was determined with a Beckman pH meter. The experiment was a 2^2 factorial, with the two diluters each containing spermatozoa at two concentrations: *(a)* 15×10^6 spermatozoa per ml. and *(b)* averaging 180×10^6 per ml. as a result of diluting at the rate of 1:9, a level of dilution employed by Kampschmidt *et al. (2, 3)*. Semen from individual ejaculates was subjected to all four treatments.

A summary of results is presented (Table 1). At a dilution level of 1:9, the progressive motility of spermatozoa in YGC was a little lower than in YC, but not significantly. At a concentration of 15×10^6 total spermatozoa per ml., motility was significantly lower in YGB than in YC ($P < 0.01$) after four or ten days. Only at four days was the interaction between diluter and concentration of spermatozoa significant ($P < 0.05$). When semen was diluted 1:9, the pH of YGB dropped from the initial level of 7.26 to 6.81, a level close to optimum for spermatozoa and close to that of YC.

Experiment III. Survival of spermatozoa in YGB with sulfanilamide. This experiment was designed to determine if the addition of sulfanilamide, which inhibits metabolism of spermatozoa *(1, 5)*, would improve livability of spermatozoa at high dilutions in yolk-glucose-bicarbonate.

The diluters compared in this experiment were prepared in the same manner as in the previous experiments, and the general procedures were also similar. The experiment was also a 2^2 factorial. The YC and YGB diluters, each with and without sulfanilamide, were compared. When included in a diluter the

TABLE 1

Progressive motility of spermatozoa and pH of semen after storage in yolk-citrate and yolk-glucose-bicarbonate, when diluted 1:9 and when diluted to have 15×10^6 spermatozoa per milliliter[a]

Storage period	Yolk-citrate		Yolk-glucose-bicarbonate	
	1:9	15×10^6	1:9	15×10^6
(days)	Progressive motility *(%)*			
0	52	52	52	52
2	54	47	49	43
4	52	40	46	16
10	36	27	28	6
	Samples with no motility *(No.)*			
10	0	2	1	10
	(pH)			
0	6.77	6.77	7.26	7.26
10	6.53	6.83	6.81	7.69

[a] Data from Experiment II. Each datum is an average of 12 observations.

sulfanilamide was at a level of 0.3%. Motilities were observed only after storage for seven days. Spermatozoa were added to all treatments at a concentration of 15×10^6 total cells per milliliter.

A summary of the data is given (Table 2). Progressive motility of spermatozoa in YGB-sulfa was slightly better than in either of the YC diluters. Motility in YGB without sulfa was, however, markedly inferior ($P < 0.01$). The interaction between diluters and sulfanilamide treatment was highly significant ($P < 0.001$). The drop in pH of YGB during storage was practically the same, whether or not sulfanilamide was present, and pH remained at a level where it was toxic in the absence of sulfanilamide.

DISCUSSION AND CONCLUSIONS

The data presented above indicate that satisfactory livability of spermatozoa can be expected with YGB at a dilution level of 1:9. The high concentration of spermatozoa enables them to increase the concentration of carbon dioxide and lactic acid sufficiently to reduce the pH to nontoxic levels. But when numbers of spermatozoa are reduced to 15×10^6 per ml., survival is extremely poor. Here the concentration of spermatozoa is so low that pH of YGB remains at a toxic level. Sulfanilamide apparently neutralizes this toxic effect.

It might be hypothesized, therefore, that pH at the levels prevailing with YGB and spermatozoa at 15×10^6 per ml. are detrimental to spermatozoa, because of excessive stimulation of metabolic activity during storage. Sulfanilamide counteracts this stimulatory effect. This hypothesis is suggested by the observations by Kinney and Salisbury *(4)*, that oxygen uptake of spermatozoa increases with increase in pH, and by Knodt and Salisbury *(5)* and Branton and Prather *(1)*, that sulfanilamide inhibits metabolism.

Swim and Parker *(8)* have stated that, since the origin of tissue culture sodium bicarbonate has been the principal buffer in media for cultivation of mammalian cells in vitro, and that "adequate control of the pH of such cultures

TABLE 2

Motility of spermatozoa and pH of semen after storage in yolk-citrate and yolk-glucose-bicarbonate, each with and without sulfanilamide[a]

Storage period	Yolk-citrate		Yolk-glucose-bicarbonate	
	Sulfa	No sulfa	Sulfa	No sulfa
(days)	Progressive motility *(%)*			
0	59	59	59	59
7	25	27	28	10
	Samples with no motility *(No.)*			
7	6	1	1	18
	(pH)			
0	6.80	6.82	7.66	7.67
7	6.67	6.68	7.55	7.56

[a] Data from Experiment III. Each datum is an average of 23 observations.

introduces a number of technical problems, for it is necessary to maintain a specified carbon dioxide tension in the atmosphere by employing gassing procedures and closed culture flasks."

Observations in the experiments described in the present paper are in agreement with the statements by Swim and Parker. In addition, an experiment has been carried out by the present authors with bull semen in YGB in large and small tubes. The large tubes were only one-third full, and the small tubes were completely filled. Prior to storage, the diluted semen was adjusted with carbon dioxide to pH 6.89. After storage for ten days, the pH in the small tubes averaged 6.93, but in the large tubes had risen to 7.20, on the average.

In the various experiments carried out by this laboratory, there has been no evidence indicating significant superiority of yolk-glucose-bicarbonate over yolk-citrate, as reported by Kampschmidt *et al.* One possible reason for this disagreement may be differences in tonicity. In yolk-citrate employed by Kampschmidt *et al. (2, 3)*, the sodium citrate solution was equivalent to 3.7% of the dihydrate salt. The buffer in the studies reported in the present paper was 2.9% sodium citrate dihydrate. This concentration has been demonstrated to be optimum by Salisbury *et al. (6)* and Swanson *(7)*. The latter observed lower livability with a 4% solution than with a 3% solution.

SUMMARY

Three experiments on livability of spermatozoa are reported. The first demonstrated that livability of spermatozoa in yolk-glucose-bicarbonate is inferior to that in yolk-citrate, when spermatozoa are at a concentration of 15×10^6 per milliliter. The second showed that there was no such difference with a dilution of 1:9, which gave an average of 180×10^6 spermatozoa per milliliter. With the lower number of spermatozoa in yolk-glucose-bicarbonate, the pH remained above 7:00, but with a dilution level of 1:9, the pH after storage for ten days was 6.81. In the third experiment, survival of spermatozoa in yolk-glucose-bicarbonate with sulfanilamide and with 15×10^6 spermatozoa per milliliter was equal to that in yolk-citrate.

It is hypothesized that metabolism of spermatozoa at the lower concentration in yolk-glucose-bicarbonate is stimulated by the high pH, but that sulfanilamide reduces or prevents this stimulation.

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STUDIES IN THE RIPENING OF LIMBURGER CHEESE. I.

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The objective of this work was to determine the ripening agents, their surface and subsurface effects, and differences in the amino acids of Limburger and Brick cheese during ripening. It was found that ripening proceeded more rapidly in the rind than in the interior of the cheese. The same amino acids were present at the same time in both the rind and the interior, but a greater concentration was found in the rind. Bacterium linens hydrolyed proteins quicker than other agents in Limburger cheese, and was responsible for the rapid ripening of the exterior. After Limburger cheese was wrapped, free amino acids increased; whereas, after Brick cheese was waxed, the rate of liberation of amino acids decreased. There was no correlation between any amino acid and the flavor of either Limburger or Brick cheese.
Editor.

Chemical reactions that occur during ripening of cheese are responsible for the changes in body and texture, as well as for the formation of compounds that give cheese its unique flavor characteristics. These reactions are initiated by enzymes present in the milk, added in the rennet extract, or produced by bacteria that survive pasteurization or are added during the process of manufacture or ripening.

Extensive bacteriological studies have been made on Limburger cheese. Kelly *(5)*, Kelly and Marquardt *(6)*, and Yale *(15)* contributed much to our understanding of the sequence of microbial changes occurring on the surface of Limburger cheese. Yale *(14)* also studied problems arising from the use of pasteurized milk in making this variety. Purko *et al. (12)* explained the associative action of yeast and *Bacterium linens* in the smear, as being due to the simultaneous secretion of growth factors and reduction in curd acidity by the yeasts.

Limburger cheese belongs to the group that is classified as surface-ripened. Kelly and Marquardt *(6)* showed that the outer rind developed a progressively higher pH as compared with the interior portion, and that it also became soft and pliable while the center remained firm. Friedman *et al. (3)* characterized an enzyme system produced by *B. linens*, which functioned at the pH of the cheese during ripening.

Since a study of chemical changes occurring in Limburger cheese during ripening had not been made, the present investigation was undertaken to compare the rate of ripening on the surface with that in the interior, by making quantitative amino acid determinations in each area. The following questions needed answering:

(1) Are the organisms of the smear the only ripening agents?
(2) Does ripening occur only at the surface?

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(3) Is there a qualitative as well as quantitative difference in amino acids at the surface and in the interior?

(4) Is there a difference in amino acid composition during the ripening of Limburger and Brick cheese?

(5) Can flavor of cheese be correlated with certain amino acids?

EXPERIMENTAL PROCEDURE

A. Cheese manufacturing methods. Limburger cheese was made from mixed milk of the University herd, which was clarified, standardized to from 3.6 to 3.7% fat, pasteurized at 143° F. for 30 min., and cooled to the setting temperature of 86° F. Five-tenths % of mixed lactic starter culture was added and incubated for one hour. Rennet extract was added at the rate of 4 oz. per 1,000 lb. milk. The remainder of the procedure was in accord with good commercial practices, and the finished cheese contained approximately 45% moisture. The curd at completion of cooking was poured into Brick cheese hoops $5 \times 6 \times 10$ in. for draining and pressing. After four to five hours of pressing at room temperature (from 75 to 80° F.), the cheese was in blocks which weighed approximately 5 lb. When the pH of the curd reached 5.2, the cheese was salted in a room which was maintained at approximately 55° F. Both dry and brine salting were practiced on different lots. The finished cheese contained approximately 2.0 to 2.25% sodium chloride.

Immediately after being salted, the blocks of cheese were lightly inoculated with a culture of yeast that had been grown in a tryptone (0.5%) and glucose (1%) broth at 22° C. The yeast had originally been isolated from the surface of good quality commercial Limburger cheese. After 48 hr., the blocks were cut into five pieces weighing approximately 1 lb. each. After all mechanical openings were closed, the cheese surface was inoculated with a culture of *B. linens* (ATCC 9174) grown in sterile milk at 22° C.

At regular intervals, the cheese was turned and rubbed with a weak saline solution until a good surface growth had developed. Approximately three weeks after manufacture, the cheese was wrapped in the conventional manner, with an underwrap of parchment, followed by one of waxed paper, and finally an overwrap of aluminum foil.

The cheese was cured at from 50 to 52° F. Samples were taken at regular intervals for analyses and judging.

B. Preparation of sample for chromatographic analysis. A 50-g. sample of cheese was dispersed in 250 g. of distilled water at 50° C. for ten minutes in a Waring blendor. The mixture was transferred to a Pasteur-Chamberland ultrafilter and filtered through a porcelain candle under 80-lb. air pressure. The first 30 ml. of filtrate was discarded and a sample was collected in a 50-ml. volumetric flask containing 25 ml. 95% ethanol. This sample was ready for application to the chromatostrip. Because of the alcohol concentration, the sample could be stored in a refrigerator for several days without deterioration. The pH of the filtrate did not vary significantly from the pH of the original sample.

Milk cultures were filtered without dilution, but the filtrate was diluted with an equal volume of 95% ethanol. Hydrogen ion measurements were made by using a Beckman Model H2 pH meter.

C. Chromatographic analysis. The amino acids were resolved on descending unidimensional paper strip chromatograms, according to the buffered filter paper technique described by McFarren *(8)*. Two buffered solvent systems were used at different pH levels; i.e., phenol buffered to pH 12 and *p*-cresol buffered at pH 2.0 and also at pH 6.2. With the strip and phenol buffered to pH 12, aspartic, glutamic, serine, glycine, threonine, and alanine were resolved. At pH 2, alanine, tyrosine, hydroxyproline, valine, methionine, norleucine, and phenylalanine were separated. At pH 6.2, alanine, tyrosine, methionine, histidine, phenylalanine, and valine were separated. After Rolland and Gross *(13)* published their procedure, which used 2-butanol and 3% ammonia (3:1) as the solvent, it was used for separating lysine, arginine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, and phenylalanine.

Samples of the standard amino acids were applied with a micropipette (Microchemical Specialties Co.) and the cheese filtrate with a Breed and Brew 0.01-ml. pipette.

D. Quantitative estimation. Quantitative estimation was made of an amino acid by eluting it from the developed spot into a buffered solution of ninhydrin, and then relating optical density of the solution as determined by a spectrophotometer to concentration of the amino acid. The procedure followed was a modification of that used by Honer and Tuckey *(4)*. Polson *et al. (11)*, Naftalin *(10)*, Awapara *(1)*, and Landua and Awapara *(7)* had previously published elution procedures for quantitative estimation of amino acids by using spectrophotometric measurements.

The following is a description of the quantitative procedure:

(1) Strips of Whatman #1 filter paper were cut 7×22 in., buffered, and dried at room temperature.

(2) Two lines, A and B, were drawn 8 and 10 cm., respectively, from one end.

(3) Six points were located on Line B, beginning one inch from one edge. At Points 1, 2, and 3, the cheese filtrate was applied in such amount that the amino acids were present in approximately 10, 20, and 10 γ concentration, respectively. In addition, at Point 3, 10 γ of standard amino acids were applied as internal standards. At Points 4, 5, and 6, amino acids were applied as external standards in concentrations of 5, 10, and 20 γ, respectively. The amino acids used depended on the buffered paper and solvent used for separation. By using internal as well as external standards, it is possible to secure a double check on the quantity of each amino acid present in the unknown mixture.

(4) After the strips were developed for the correct length of time, they were dried at room temperature for two hours in a current of flowing air. If phenol or *p*-cresol was used as a solvent, the strips were immersed in ethyl ether and redried.

(5) The strips were then dipped into a solution of 0.1% ninhydrin in ethanol to locate the amino acids. The strips were dried two hours at room temperature. If the papers were buffered to pH 12, 4% glacial acetic acid was added to the ninhydrin solution, to lower the pH of the paper within the range 5 to 6, which is optimum for color development *(2, 9)*.

(6) The Rf values of the different amino acids were calculated, and the chromatostrip was cut into six 1-in. strips at points equidistant betwen sample application spots.

(7) The amino acid spots were cut off, transferred to a numbered test tube with plastic-tipped forceps, and forced to the bottom with a glass rod.

(8) Control blanks were prepared from each strip, by cutting a piece of appropriate size from an area where there was no color development and treating it in the same manner as the amino acid spots.

(9) Five-tenths ml. of 6.2 buffer and 0.5 ml. of 2% ninhydrin solution were added to each tube so that each paper strip was saturated. The tubes were stoppered and placed in the dark for two hours. Then 3 ml. of distilled water was added and the contents were mixed.

(10) The optical density values were read at 570 mμ with a Beckman DU spectrophotometer.

(11) Standard curves were drawn for each amino acid, and the concentration of the unknowns was calculated by use of the standard curves and Strip 3, which contained the internal standards.

RESULTS AND DISCUSSION

To determine the relative contribution made to ripening of cheese by each of the agents during the manufacture and curing periods, separate systems were studied. The following cultures and agents, added in the amount used in the manufacture of cheese, were inoculated into 100-ml. samples of sterilized skimmilk in 500-ml. Erlenmeyer flasks: *B. linens*, 1% (9174 ATCC); yeast culture, 0.5%; mixed lactic starter culture, 0.5%; starter culture (0.5%) + rennet extract (4 oz/1,000 lb.) + sodium chloride (2%). The samples were placed on a Burrell Model BB wrist-action shaker in a refrigerated room maintained at approximately 60° F. and shaken continuously for varying periods of time. The samples were analyzed for a limited number of amino acids by paper strip chromatography, to determine whether the casein as it existed in the sterilized skimmilk could be attacked by the enzymes in the different systems. Results of the experiment are given (Table 1).

The data show that free amino acids were liberated in all systems studied, with the greatest concentration being in the cultures of *B. linens;* whereas, the lowest was in the yeast and starter culture systems. Sodium chloride seemed to accelerate hydrolysis, as compared with starter and rennet extract alone. However, there was a similarity to the pattern in which amino acids were split off in each system. All samples showed glutamic acid, leucines, alanine, and valine. Although casein is not in the same form in cheese as it is in the sterilized skim-

TABLE 1
Amino acids liberated in sterile skimmilk shake cultures
(Incubated at 60° F. γ/ml)

Culture	9174 *B. linens*	Yeast culture	Starter culture	Starter and rennet extract	Starter + rennet + NaCl (2%)
Age (days)	12	39	7	23	52
pH	8.2	7.5	4.45	4.6	4.47
Alanine	184	+[a]	20	37	108
Aspartic Acid	765	+	+	−	+
Glutamic Acid	298	38	55	48	+
Glycine	344	+	−	−	−
Leucines	411	+	+	73	233
Methionine	285	−[b]	−	+	119
Serine	94	50	−	−	+
Threonine	133	+	14	−	+
Tyrosine	1500	−	+	+	+
Valine	475	+	+	39	68

[a] + present.
[b] − absent.

milk, nevertheless it might be assumed that the different agents would contribute to hydrolysis of casein during cheese ripening in the same order of magnitude (as shown in Table 1).

If this is true, then ripening of Limburger should occur most rapidly on the surface; furthermore, ripening should occur simultaneously in the interior, owing to the action of rennet extract, the starter culture, and perhaps the bacteria that survived pasteurization. To test this hypothesis, batches of Limburger cheese were studied by comparing the liberation of amino acids in the outer portion with that in the interior. For the rind sample, a slice of cheese ¼-in. was cut from each outer surface of one block of cheese. It was grated and mixed. A representative sample was taken for amino acid analysis and treated as described (under Experimental Procedure). The inner portion sample was taken from the center of the block and treated in the same manner. A different block or pound sample of cheese was used at each analytical period. The results are given (Table 2).

The data show that ripening occurred two to three times as rapidly in the rind portion as in the center of the cheese, when concentration of free amino acids was used as the index. If an amino acid was found in the rind portion, it was also present in the center, but in a lower concentration. Hence, the difference in amino acid pattern between rind and center was quantitative and not qualitative. The amino acids, which appeared when the cheese was only one day old, continued to increase throughout the aging period. The leucines, glutamic acid, tyrosine, valine, and alanine were present in greatest concentration.

After the cheese was wrapped, flavor development tended to occur first in the rind portion. However, no correlation with any one or any combination of amino acids with flavor formation could be found.

One reason for variation in analytical results from period to period might be the difference in individual blocks of cheese. It is reasonable to assume that different blocks would not progress in ripening at exactly the same rate.

TABLE 2

*Amino acids liberated during ripening of Limburger cheese**

Age (days)	1	11		18		32		46		80		101	
Portion	Whole	Rind [a]	Int. [b]	Rind	Int.	Rind	Int.	Rind	Int.	Rind	Int.	Rind	Int.
pH	4.95	5.25	4.90	5.65	5.0	5.95	5.00	6.2	5.45	7.0	–	7.2	–
Alanine	0.15	0.38	0.13	0.60	0.23	0.39	0.13	0.39	0.29	+	0.80	0.73	
Aspartic Acid	+[c]	+	Lost	+	+	+	+	0.32	+	0.80	+	+	
Glutamic Acid	0.24	0.47	Lost	1.11	0.21	0.81	0.08	0.62	0.44	1.26	0.96	1.96	
Glycine	–[d]	+	Lost	0.08	+	+	+	0.15	+	0.28	0.14	0.20	
Leucines	0.19	0.35	0.32	1.39	0.43	1.38	0.50	1.38	1.05	+	2.47	2.42	
Serine	–	+	Lost	0.29	+	0.19	+	0.12	+	0.84	0.20	0.15	
Tyrosine	+	0.52	0.12	0.14	0.19	0.32	0.92	0.58	1.32	+	0.36	0.99	
Valine	0.23	0.28	0.23	0.26	+	0.25	0.16	0.31	0.30	+	0.40	0.71	

* Mg/g of cheese.
[a] Rind – ¼-in. cut from outer surface.
[b] Interior – center portion.
[c] + present.
[d] – absent.

Brick cheese also is a surface-ripened cheese, but it has a lower moisture content and is waxed instead of wrapped. The flavor of Brick cheese is related to, but different from, that of Limburger cheese. Two lots of Brick cheese and one of Limburger were made in order to study the amino acid patterns. The same cultures of starter and smear organisms were used for both varieties. At approximately the third week, the Brick cheese was waxed and the Limburger wrapped and then aged in the same room at from 50 to 52° F.

Results of analyses for 20 amino acids in one lot of Brick and the batch of Limburger for a ten-week period are shown (Tables 3 and 4). Since the amino acid patterns of the two lots of Brick cheese are similar, data on only one lot are given.

TABLE 3

Amino acids in Limburger cheese 124[a]

Age (weeks)	0	2	4	5	6	8	10
pH	5.10	5.5	5.5	5.6	5.6	5.6	5.6
Portion		whole	whole	whole	whole	whole	whole
Amino Butyric Acid		+			0.11	0.23	0.15
Aspartic Acid		+	0.18	0.42	0.46	0.43	0.45
Arginine			+	+	++	++	++
Alanine		0.11	0.60	0.62	0.81	0.89	0.89
Cystine					0.45	0.45	0.45
Glutamic Acid		0.56	0.88	1.00	2.81	1.68	2.58
Glycine		+	+	+	0.37	0.21	0.38
Histidine			+	+	0.11	0.11	0.26
Hydroxyproline		0.17	0.10	0.11	0.13	0.11	0.17
Leucines		0.34	0.95	1.10	2.20	2.80	1.90
Lysine		+	0.18	0.20	0.21	0.19	0.37
Methionine		0.20	0.63	0.84	1.02	1.10	1.10
Norleucine					0.10	0.11	0.45
Phenylalanine		+	+	+	0.45	0.42	0.10
Proline				0.10	0.23	0.29	0.32
Serine				0.10	0.19	0.32	0.29
Threonine				0.19	0.59	0.59	0.60
Tyrosine		0.10	0.64	0.80	0.88	0.87	0.89
Tryptophan		+	+	+	+	+	+
Valine		0.10	0.10	0.27	2.20	2.52	1.81

[a] Mg/g cheese.

The data show a higher concentration of free amino acids in Limburger than in Brick, as might be expected, but the degree of difference in some of the amino acids is surprising. This is particularly true for valine, which is lower in the Brick cheese. Glutamic acid, methionine, threonine, tyrosine, and valine all show at least 100% greater concentration in Limburger at the end of ten weeks than in the lot of Brick. Hydroxyproline is much higher in the Brick cheese than in Limburger. The differences in concentration of the amino acids became strikingly apparent after the lots were waxed and wrapped. The amino acids, which had attained a high concentration in Limburger before it was wrapped, continued to increase after the cheese was wrapped, but this did not occur with Brick cheese after it was waxed. On the other hand, the rate of liberation of many of the amino acids was markedly decreased after the Brick cheese was waxed. Nevertheless, the unique flavors of each variety could not be correlated with the presence or absence of any single amino acid or group of amino acids.

TABLE 4

Amino acids in Brick cheese 1120[a]

Age (weeks)	0	1	2	4	6	8	10
pH	5.4	5.55	5.45	5.5	5.5	5.5	5.5
Portion		whole	whole	whole	whole	whole	whole
Aspartic Acid	+	++	0.05	0.11	0.40	0.50	0.43
Arginine			+	+	0.53	0.49	+
Alanine		+	0.18	0.24	0.85	0.52	0.46
Cystine				+	+	+	++
Glutamic Acid	+	0.11	0.22	0.35	0.36	0.77	0.60
Glycine	+	0.90	+	0.32	0.41	0.50	0.50
Histidine			0.11	0.11	0.29	+	+
Hydroxyproline		+	0.11	0.44	0.55	0.60	0.78
Leucines		++	0.20	0.78	0.87	0.90	1.10
Lysine					+	+	+
Methionine			+	0.13	0.45	0.46	0.48
Norleucine			+	+	++	0.11	0.10
Phenylalanine		+	+	+	+	0.46	0.52
Proline			+	++	++	0.11	++
Serine			+	+	++	0.31	0.21
Threonine	+	+	+	+	++	0.11	0.18
Tyrosine		0.13	0.09	0.12	0.11	0.23	0.42
Tryptophan				+	+	+	+
Valine	+	+	0.10	0.21	0.47	0.49	0.19

[a] Mg/g cheese.

SUMMARY

Ripening of Limburger cheese was studied by determining the quantity of individual amino acids liberated throughout the aging period.

Results of the study show that:

Ripening proceeds more rapidly in the rind portion than in the interior, but not exclusively in this area.

The difference in amino acids in the rind and in the center represents a quantitative rather than a qualitative relationship, for the same amino acids were present in both areas at essentially the same time, but in a higher concentration in the rind.

Bacterium linens was able to hydrolyze milk proteins more rapidly than other agents used in the manufacture and ripening of Limburger cheese. The surface of the cheese was heavily inoculated with a culture of these organisms and, therefore, *B. linens* was probably responsible for the rapid ripening of the exterior.

In the lots of Brick and Limburger cheese studied, there was a quantitative and a qualitative difference in the amino acid patterns at the end of ten weeks of aging. After Limburger cheese was wrapped, the free amino acids continued to increase in concentration; whereas, after the Brick cheese was waxed, the rate of liberation of amino acids was reduced as compared with Limburger. Hydroxyproline was markedly higher in concentration in the Brick cheese than in the Limburger.

No correlation between the presence of any single amino acid and characteristic flavor development in Limburger or Brick cheese was established during the aging period by organoleptic judging of the samples.

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OCCURRENCE OF MULTIPLE HEMOGLOBINS IN CERTAIN BREEDS OF DAIRY CATTLE[1]

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The occurrence of multiple adult hemoglobins in dairy cattle was investigated by examining the blood by paper electrophoresis from 158 animals. All Holsteins, Brown Swiss, and Ayrshires had only Hemoglobin A, which was found also as the sole hemoglobin component in the blood of eight Jerseys and 19 Guernseys. It was characteristic of Holsteins. A faster moving component, Hemoglobin F, was found with Hemoglobin A in the blood of 13 of 34 Guernseys and 13 of 25 Jerseys; whereas, two Guernseys and four Jerseys had only Hemoglobin F. The ratio of A to F was 1:1 for all blood samples containing both types. *Editor.*

The occurrence of multiple hemoglobins in the adults of some animal species has recently been reported by several investigators. Interest in this subject was stimulated by the demonstration of abnormal hemoglobins in man, by Pauling *et al. (7)*, and by the recent development of paper electrophoresis. By use of this technique, Harris and Warren *(5)* found two hemoglobins in 18 of 72 goats and in five of 12 sheep. Giri and Pillai *(4)* found two hemoglobins in rat and buffalo blood, but only one in the blood of cattle, sheep, goats, horses, dogs, rabbits, and guinea pigs. Sydenstricker *et al. (10)* reported single hemoglobins in dogs and rabbits, two hemoglobin components in turtles and chickens, and suggestive evidence of inhomogeneity in hemoglobin preparations from rats and alligators. Two hemoglobin components were observed in mature chickens by Johnson and Dunlap *(6)*. Rodnan and Ebaugh *(8)* found homogeneous hemoglobins in all mammals tested (cow, sheep, goat, llama, chimpanzee, rhesus monkey, pig, cat, mouse, rat, hamster, macaque, guinea pig, dog, and rabbit), except the horse and one of five sheep. Saha, Dutta, and Ghosh *(9)* reported single hemoglobins in cattle, goats, and rabbits.

Cabannes and Serain *(2)* were the first to report two electrophoretically distinct hemoglobin components in cattle. Sixty-four of the 80 animals they examined had a single hemoglobin, 15 had a second, faster-migrating component as well, and one possessed only the second component. The cattle used in this study were of mixed breeds, the progeny of European bulls and Algerian cows. The presence of the second hemoglobin was not associated with disease, nor were the data sufficient to establish genetic control.

The results of Cabannes and Serain *(2)* indicate the necessity of investigating an adequate number of individuals, and also suggest that breed differences might exist. The present investigation was initially concerned with determining whether multiple adult hemoglobins normally occur in dairy cattle, and later was concerned with the question of breed differences in this respect.

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EXPERIMENTAL PROCEDURE

Animals. Hemoglobin preparations from 158 animals were examined. This number included 79 Holsteins, 25 Jerseys, 34 Guernseys, eight Ayrshires, eight Brown Swiss, and four crossbreds. Ages ranged from four months to 14 years. Calves younger than four months were not included because, according to unpublished data in this laboratory, fetal hemoglobin persists in the blood of some calves to about 110 days of age. All animals used in this study were apparently healthy.

Paper electrophoresis. The hemoglobin solutions used for analysis were prepared from heparinized blood, by the method of Drabkin *(3)*. No evidence of proteins, other than hemoglobin, was found on any of the completed strips. The hemoglobin concentration was adjusted to 4% before analysis.

The apparatus used for paper electrophoresis was a ridge-pole type cell, the Spinco Model R. Of the buffers tried in preliminary experiments, a barbital buffer of pH 9.0, ionic strength 0.1, was found to be most suitable. A constant direct current of 10 ma. was applied to the eight Whatman No. 3 MM paper strips for 16 hr. Most runs were carried out in a refrigerator at 4° C., rather than at room temperature. Use of the low temperature and high ionic strength repressed spreading and trailing, and resulted in better resolution than could be obtained at room temperature and low ionic strength. Sample volume was usually 5 μl. applied in a strip across the paper, but in some cases in which qualitative runs using 1-μl. spots indicated the presence of a single component, 3-μl. samples were used. Preparations containing two components were usually analyzed in duplicate.

At the end of electrophoresis the paper strips were dried, stained with bromophenol blue, rinsed and dried by the procedure recommended by Block, Durrum, and Zweig *(1)*. Strips containing two components were quantitatively evaluated by means of an automatic recording and integrating densitometer (the Analytrol, manufactured by the Spinco Division, Beckman Instruments, Inc.).

RESULTS AND DISCUSSION

A single hemoglobin component was found in blood samples from all the Holsteins, Brown Swiss, Ayrshires, and a pair of crossbred twins, predominantly Guernsey. This hemoglobin, hereafter referred to as Hemoglobin A, migrated about 2.4 cm. toward the anode, under the conditions used. The number of Holsteins tested seemed sufficient to establish that a single adult hemoglobin is characteristic of this breed.

In contrast, a second easily distinguished hemoglobin component was found in the blood of a large percentage of Jersey and Guernsey cattle. The second component, designated Hemoglobin F for reasons to be given in a subsequent communication, migrated about 3.9 cm. toward the anode. Both components were found in 13 of 34 Guernseys and 13 of 25 Jerseys. Both members of a set of Holstein-Jersey twins also possessed both hemoglobins. Nineteen Guernseys and eight Jerseys had only Hemoglobin A, and two Guernseys and four Jerseys

TABLE 1

Distribution and migration distances of Hemoglobins A and F in the blood of dairy cattle

Breed	Distribution			Migration distance[a]	
	Hemoglobin A only	Hemoglobin F only	Hemoglobins A and F	Hemoglobin A	Hemoglobin F
				(cm.)	*(cm.)*
Holstein	79	0	0	2.4 ± 0.16[b]	
Brown Swiss	8	0	0	2.5 ± 0.08	
Ayrshire	8	0	0	2.4 ± 0.13	
Crossbred[c]	2	0	0	2.5	
Guernsey	19	2	13	2.4 ± 0.18	3.9 ± 0.10
Jersey	8	4	13	2.5 ± 0.18	3.9 ± 0.16
Holstein-Jersey	0	0	2	2.4	3.8

[a] Means with standard deviations.
[b] Mean value for 17 animals. Conditions of electrophoresis for the remainder were different from standard.
[c] Predominantly Guernsey.

possessed Hemoglobin F alone. These results are summarized (Table 1), together with the distance of migration of each component. The latter data indicate that Hemoglobin A is probably identical in the five breeds, since the slight differences in mobility are not significant. Similarly, the Hemoglobin F of the Guernseys is probably the same as that of the Jerseys.

Hemoglobin F occurred in both sexes with about equal frequency. Included in the 34 Guernseys were 16 mature bulls, nine of which had Hemoglobin A only, one Hemoglobin F only, and six, both A and F. The corresponding figures for nine Jersey bulls are three with A only, none with F alone, and six with both types.

Typical paper electrophoretic patterns of samples containing Hemoglobin A only, Hemoglobin F only, and Hemoglobins A and F together, are reproduced (Figure 1).

The mean relative concentration of Hemoglobin A in samples containing both types are given by breed (Table 2). To obtain these values, a correction was applied to account for the hemoglobin adsorbed to the paper along the path of migration. Experiments to be reported elsewhere showed that the leading component, Hemoglobin F, contributes virtually all of the adsorbed protein, and that the correction is not only valid but also necessary. According to the results (Table 2), the ratio between the two components is close to 1 : 1. The range of

TABLE 2

Relative concentration of Hemoglobin A in blood samples containing both Hemoglobins A and F

Breed	Range	Mean	Standard deviation
		(%)	
Guernsey	48.9-55.2	51.8	± 2.04
Jersey	50.1-56.3	52.6	± 1.82
Holstein-Jersey	49.1-55.9	52.5	

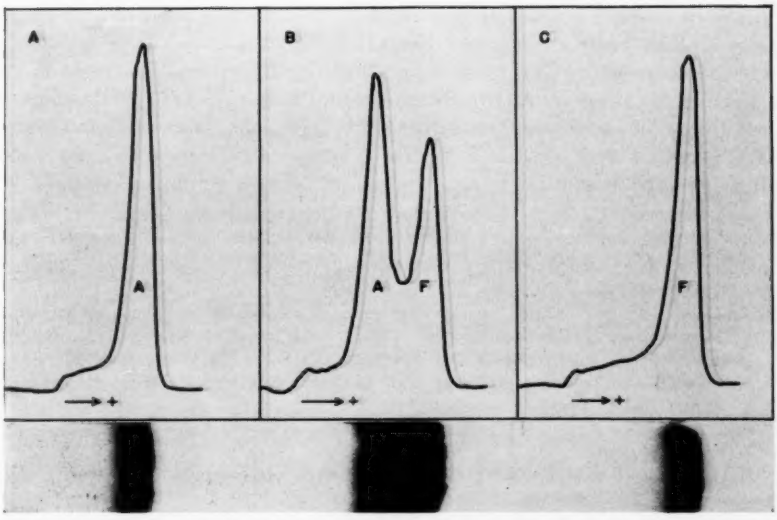

FIG. 1. Examples of the three types of paper electrophoretic patterns and tracings obtained from hemoglobin preparations from cattle. The starting point is indicated by the tail of the arrow in each case. Electrophoretic conditions are described in the text.

A. Hemoglobin A; B. Hemoglobin A and F; C. Hemoglobin F.

values is narrow, and the difference between the mean values for the two breeds is of doubtful significance ($P = 0.03$).

The occurrence of Hemoglobin F in some adult cattle appears to be a breed-specific phenomenon. Although a few instances of close relationship between pairs of animals are included, the Holstein, Jersey, and Guernsey samples were sufficiently random to support this conclusion. The Brown Swiss and Ayrshire samples were perhaps not large enough to allow confident classification of these breeds. The cattle used by Cabannes and Serain *(2)* were of mixed breeds, and these authors were therefore unable to determine whether breed specificity existed. Hemoglobin F is almost certainly not pathological in origin, since all the animals used in the present study were apparently healthy. Moreover, neither constancy of ratio between the concentrations of the two components nor breed specificity would be expected if Hemoglobin F were of pathological origin.

Some of the results obtained so far suggest genetic control of the hemoglobin type in cattle. An investigation of this possibility is now being carried out.

SUMMARY

An investigation was conducted to determine whether multiple adult hemoglobins occur normally in dairy cattle. Hemoglobin preparations from 158 animals were examined by paper electrophoresis.

All the Holsteins, Brown Swiss, and Ayrshires examined possessed a single component, designated Hemoglobin A. It was concluded that the possession of

Hemoglobin A alone is characteristic of Holsteins. On the other hand, a second, faster-migrating component, called Hemoglobin F, was found in the blood of 15 of 34 Guernseys and 17 of 25 Jerseys. Nineteen Guernseys and eight Jerseys had Hemoglobin A only, and two Guernseys and four Jerseys had Hemoglobin F alone. In all blood samples containing both types, the ratio of Hemoglobin A to Hemoglobin F was close to 1:1. The occurrence of Hemoglobin F in some adult dairy animals appears to be a normal, breed-specific phenomenon.

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FACTORS INFLUENCING METABOLIC ACTIVITY OF BULL SPERMATOZOA. III. pH

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Effects of pH on respiration, on aerobic glycolysis of spermatozoa, and on the calculated respiratory quotients were studied. The pH levels of the saline-phosphate diluents varied from 5 to 8. Aerobic fructolysis varied several fold, and directly with pH. The oxygen consumption of the cells was not markedly influenced by pH. However, a significant pH × ejaculate interaction was found for each metabolic measure, except for the R. Q. values, of which a large part of the variance was associated with individual ejaculates. *Editor.*

Experiments in our laboratory have emphasized that not only are aerobic conditions frequently met with in the handling and storage of semen for artificial insemination *(12)*, but that a wide variety of conditions influence the extent and nature of the aerobic metabolism of bovine spermatozoa. The rate of aerobic utilization of fructose by spermatozoa collected from some bulls in the summer months is significantly less than is that by spermatozoa collected from the same bulls in the winter months, though the utilization of oxygen remains nearly the same *(8)*. Oxygen utilization per unit number of washed bull spermatozoa can be made to vary over relatively wide ranges by varying the concentration of cells *(2)* and by use of suspension media containing varying concentrations of phosphate, citrate, chloride *(3)*, or bicarbonate *(11)* anions. The physiological age of the sperm cells appears to alter the type of substrates utilized, as reflected by the respiratory quotients observed for both human *(13)* and bull spermatozoa *(1)* in their own seminal plasma.

The dependence of most enzyme systems on pH optima is well known and the interrelation of pH and respiration rate of washed bull sperm cells has been studied *(6)*. However, the extreme pH ranges investigated usually have resulted in the use of different anions in the buffers, which might confound the observed effect of pH on the respiratory rates. Therefore, this paper reports a reappraisal of the effect of pH on the respiration. In addition, it reports the effect of pH on the aerobic glycolysis, calculated respiratory quotients, and livability at 37° C., of bull spermatozoa in semen diluted 1:4 with a diluent composed of one-half Na, K, and Mg chlorides and one-half composed of a buffer made up solely of sodium salts of phosphoric acid.

Under such circumstances, the experiments show that the optimum pH for respiration varies greatly from ejaculate to ejaculate, but tends to be highest at the higher pH used. The optimum pH for motility is about that for oxygen uptake, the rate of fructose utilization and of lactic acid accumulation varies directly with pH, but the calculated R.Q. values do not. At the higher pH's

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more lactic acid is produced than can be accounted for solely on the basis of fructose disappearance. In addition, the results prove that the rate of fructose utilization is greater in the presence of the CO_2 evolved from respiration than it is when the evolved CO_2 is absorbed by KOH.

METHODS

The methods of semen collection, chemical analyses, motility estimations, pH determinations, and respiration studies have been reported *(5)*. Respiratory quotients were determined by the direct method of Warburg *(14)*, in which three 5-ml. Warburg flasks were used for each observation; one for determinations of the initial levels of substrates in the diluted semen and of bound CO_2, one containing 20% KOH in the center well for the measurement of O_2, and one containing no KOH for the measurement of evolved CO_2 and CO_2 retention by the buffer. The single side arm of two flasks contained 0.1 or 0.2 ml. 3*N* sulfuric acid, which was tipped into the flask contents after temperature equilibration in the one flask from which initial values were obtained, and at the end of the two- or four-hour incubation period in the other flask containing no KOH. In the third flask, the reactions were stopped a few minutes later, after motility estimations and pH determinations were made on the flask contents. Chemical analyses were made on the contents of each flask.

The semen was diluted 1:4 with diluents having an initial pH varying from 5 to 8. Because of the buffer capacity of the semen itself, the initial pH values of the mixed semen and diluent did not extend over this extreme range. However, the buffers maintained pH without marked changes throughout the incubation periods (see Table 4). The diluents were composed one-half of an isotonic solution of chloride salts and one-half of an isotonic mixture of monobasic sodium phosphate, 0.17 *M*, and dibasic sodium phosphate, 0.13 *M*, combined in such proportions as to give the appropriate pH. The combinations required for diluents of the stipulated pH's are shown (Table 1).

TABLE 1
Composition of buffered diluents

		pH			
0.8 g. NaCl		5	6	7	8
		(%)			
0.04 g. KCl	3.48% $Na_2HPO_4 \cdot 7H_2O$	2.5	20.0	71.5	96.0
1:1					
0.04 g. $MgCl_2 \cdot 6H_2O$	2.35% $NaH_2PO_4 \cdot H_2O$	97.5	80.0	28.5	4.0
Distilled water to 100 ml.					

The molarity of the phosphate ion in these final solutions varied from 0.084 at pH 5 to 0.066 at pH 8. This is somewhat higher than the level of 0.05 *M* phosphate, earlier found to be the minimum level inhibitory of respiration *(10)*, but is much lower than the level found to have a maximum depression on respiration *(3)*.

RESULTS

The first experiment was designed to determine the livability at 37° C. of spermatozoa suspended at seven different pH levels under two conditions. In one, 1.0 ml. of diluted semen was placed in 10 × 75 mm. test tubes, resulting in only partially aerobic conditions, for the surface area to volume ratio was only 0.5 *(12)* and the tubes were not shaken during incubation. In the other condition, the 1.0 ml. of diluted semen was placed in 5.0-ml. Warburg flasks and the contents shaken at the rate of 110 strokes per minute, resulting in complete aeration and a surface area to volume ratio somewhat greater than the measured ratio of 1.71 at the gas-liquid interface. The initial pH of the nine ejaculates averaged 6.4. The mean cell count per treatment was about 200×10^6 cells per ml.

The respiration measurements were terminated at the end of two hours, when final motility and pH estimations were made. The time required to complete these procedures made it impossible to terminate the test-tube experiments at the same time. Arbitrarily, it was decided to determine motility at one-half hour intervals and to terminate these studies when the motility reached zero for any pH level. The cessation of motility invariably occurred first in the tubes diluted with the pH 5 diluent, and the time interval varied from 7.5 to 9.5 hr. This arbitrary decision rendered statistical analysis of the combined results impossible.

TABLE 2

Livability of spermatozoa at 37° C. in 10 × 75 mm. test tubes, and in Warburg flasks under air (mean of nine ejaculates for each value)

pH of added diluent	In unshaken test tubes			In 5-ml. Warburg flasks			
	Motility after[a] 7.5 to 9.5 hr.		Final pH	Motility after 2 hr.		Final pH	$-Z_{O_2}$[b]
	(%)	*(rate)*		*(%)*	*(rate)*		
5.0	0	0.0	5.26	14	0.7	5.58	11.2
5.5	2	0.1	5.60	16	0.7	5.86	12.2
6.0	6	0.2	6.03	22	0.8	6.05	12.0
6.5	10	0.4	6.52	29	1.6	6.51	13.2
7.0	21	1.0	6.94	36	1.8	6.99	12.9
7.5	32	2.1	7.70	34	2.1	7.41	11.9
8.0	29	1.5	7.46	32	2.0	7.66	12.6
Undiluted semen at 37° C.	12	0.3	—				
At room temp.	47	2.4	5.40				

[a] Motility estimates recorded when motility reached zero for any one treatment (pH 5 in every case).

[b] $-Z_{O_2}$=µl. O_2 uptake/10^8 cells/hr.

The mean data are shown (Table 2). The cells retained their motility better under the conditions of restricted respiration in the small storage tubes. Under both procedures, the motility was maintained better at the higher pH's. In the diluent buffered at pH 7.5, the motility at the end of 7.5 to 9.5 hr. in the test tubes was nearly the same as in the completely aerated flasks after only two hours. In comparison with that pH as a standard for each procedure, the aerated cells in the Warburg flasks at the low pH's lived better than did those in the

test tubes at the low pH's, presumably as a result of the maintenance of a somewhat higher pH.

Though the mean pH for optimum oxygen consumption was at 6.5, the highest respiration rate for one ejaculate was at pH 8.0, and for another it was at pH 5.0, resulting in a highly significant ejaculate × pH interaction.

Carbon dioxide production and R. Q. The second experiment, confined to four ejaculates, was conducted to determine the amount of CO_2 evolved in relation to oxygen consumption. Because of the volumes of semen required for the CO_2 measurements, the number of pH levels was reduced to four, buffers of pH 5, 6, 7, and 8 only being used. The results are shown (Table 3).

TABLE 3

Effect of pH on motility and respiration of diluted spermatozoa (mean of four ejaculates)

Added buffer	Final pH	Final motility		Oxygen uptake	Carbon dioxide production	R.Q.
(pH)		*(%)*	*(rate)*	$-Z_{O_2}$	$(+Z_{CO_2})$	
5.0	5.61	33	0.9	7.2	5.8	0.80
6.0	6.16	35	1.0	7.1	5.9	0.83
7.0	6.89	43	1.8	10.8	8.4	0.78
8.0	7.28	53	2.5	13.4	10.8	0.81

Here optimum livability, maximum oxygen uptake, and CO_2 production resulted from the diluent buffered at pH 8.0. The differences shown in O_2 uptake and CO_2 production at the four pH's are statistically significant, but of the R. Q. values none differs significantly from the over-all mean of 0.8.

Finally, an experiment was conducted to include not only O_2 uptake and CO_2 production, but to determine the initial pH of the semen-diluter mixtures and the fructose utilization and the lactic acid accumulation in each flask. In this fashion, one might obtain inferential information on the validity of R. Q. estimates, for the basic assumption of the direct technique of Warburg is that respiration proceeds in exactly the same way in each of the flasks used. In this experiment, nine ejaculates were used to determine the individual values at the end of two hours, and three others provided data during a four-hour incubation. In this latter series, enough semen was available to include undiluted whole semen in the study. The first nine ejaculates had an initial pH average of 6.55, with a concentration giving 225 million cells per ml. in the buffer-diluted semen; whereas, that of the three samples was an average initial pH of 5.94 and a concentration yielding a final count of 340 million cells per ml. on dilution.

The initial and final pH and motility of the spermatozoa after the incubation are given (Table 4). The data on O_2 and CO_2 exchange, R. Q., and aerobic fructolysis are presented as Z values, or the exchange per 100×10^6 cells per hour (in Table 5). While the concentration of spermatozoa found in the semen samples used in this experiment varied widely and influenced the absolute Z values for any one ejaculate, the comparative values for the treatments are valid, each ejaculate having been used for all treatments.

TABLE 4

Effect of pH on initial and final pH of the mixture, and the final motility of the spermatozoa after aerobic incubation at 37° C.

Added diluent	pH diluted semen		Final motility	
(pH)	*(Initial)*	*(Final)*	*(%)*	*(rate)*
	2-hour incubation (9 ejaculates)			
5.0	5.63	5.71	38	0.9
6.0	6.13	6.15	31	1.1
7.0	6.97	6.96	36	2.1
8.0	7.53	7.55	48	2.7
	4-hour incubation (3 ejaculates)			
5.0	5.46	5.51	17	0.2
6.0	5.93	5.95	37	0.4
7.0	6.81	6.81	37	1.3
8.0	7.32	7.28	43	1.8
Semen only	5.94	6.27	37	1.7

The O_2 consumed and CO_2 produced at pH 5 and pH 8 were significantly different ($P = < .05$) than those values for pH's 6 and 7. For the fructose consumed, the differences among pH levels were highly significant ($P = < .01$), and a highly significant pH × ejaculate interaction ($P = < .01$) was obtained. The statistical significance of these sources of variation in the lactic acid accumulation was of the same order of probability. The presence or absence of KOH in the center wells of the flasks and, hence, the CO_2 tension in the gaseous phase, had a highly significant ($P = < .01$) effect on both the fructose utilization and lactic acid accumulation, both being greater in the presence of CO_2.

A plot of the data of fructose utilization and lactic acid accumulation for those flasks in which O_2 consumption was measured, and which contained KOH so that respiration took place in the absence of CO_2, shows that the means fell on essentially straight lines, the linear regression coefficient being highly significant in each case. At pH 7 and 8, more lactic acid accumulated than fructose was utilized. There was no effect of pH on the R. Q. values obtained. However, there was great variation in mean R. Q. values from ejaculate to ejaculate, the probability being $< .001$ that the differences were due to chance.

TABLE 5

Effect of pH on respiration, R.Q. values, and aerobic fructolysis at 37° C. (mean of 12 observations)

pH added diluent	$-Zo_2$[a]	$+Z_{co2}$	R.Q.	−Z Fructose		Z+ Lactic Acid	
				$-CO_2$	$+CO_2$	$-CO_2$	$+CO_2$
	(μl.)	*(μl.)*		*(μg.)*	*(μg.)*	*(μg.)*	*(μg.)*
5	7.47	6.50	0.87	37.7	57.7	12.0	34.3
6	6.65	5.59	0.84	62.3	82.7	63.3	121.0
7	6.79	5.31	0.78	74.3	179.3	144.7	223.0
8	8.30	6.96	0.84	113.7	230.7	203.7	224.7
Semen[b] only	6.65	6.90	1.04	55.0	60.8	−12.5	−10.8

[a] Z = metabolic activity/10^8 cells/hr.
[b] Three ejaculates only.

DISCUSSION

The data published here make it clear that variations in pH have a far greater and a more standard effect on the rate of fructolysis than they do on the respiration rate of spermatozoa in diluted semen. The mean respiration tended to be highest at the higher pH levels and at levels higher than that reported by Lardy and Phillips *(6)* as optimum for washed bull spermatozoa.

However, the variability exhibited by the individual ejaculates in the pH at which maximum oxygen uptake occurred was striking. Such differences in respiration rate appear to be due to variations in the mineral components of the seminal plasma *(9)*.

The steadily increasing rate of aerobic fructolysis observed with increasing pH, from approximately 5.5 through 7.5, reflects the increased motility observed at the end of the incubation period. Many investigators have observed this effect of pH on motility, but the manifold increase in the rate of fructolysis over the pH range used had not heretofore been reported. One might infer from these data that decreasing the pH of semen would depress fructolysis and thus the motility of spermatozoa, and permit of longer livability on storage. However, with the phosphate buffers here used, no evidence to substantiate this was found. The sperm cells did not live so well at the low pH, and showed no indication of an ability to revive from the treatment.

The consistent observation that more lactic acid was produced than fructose was utilized, during incubation at pH's above 6, particularly in flasks where the CO_2 evolved from respiration was absorbed by KOH, has been observed before in our laboratory. However, the experimental conditions under which this phenomenon was observed have varied widely, and no indisputable evidence of its occurrence under essentially normal metabolic conditions has been available until now *(4)*. The data indicate that semen provides a substrate for lactic acid formation by bovine spermatozoa, apart from that provided by seminal fructose. It is tempting to speculate that the unknown substrate will be found in the α-glycerophosphocholine *(7, 15)* component of semen. However, White *(15)*, while studying the oxidation by bull spermatozoa of glycerol, and the formation of fructose and subsequent formation of lactic acid in the process, was unable to demonstrate utilization of glycerophosphate by bull spermatozoa.

The R.Q. of diluted semen appears to be a function of the semen sample itself, for 59% of the total variance of R.Q. was associated with ejaculates. Because of the effect of CO_2 evolved from respiration, on the metabolic activity of bovine spermatozoa, the direct method of Warburg *(14)* is not adequate as a means of determining the R.Q. of semen, and more refined techniques must be used.

SUMMARY

Bull semen was diluted 1 : 4 with a solution containing Na, K, and Mg chlorides, and the buffer composed entirely of Na phosphates combined to provide a pH range from 5 to 8. pH per se did not influence the respiratory quotients obtained, nor was there a marked effect of pH on oxygen uptake of the sperm cells,

though it tended to be highest at the higher pH levels. However, pH did influence markedly aerobic fructolysis; the higher the pH, the greater the rate and the greater the lactic acid produced in excess of fructose utilization. A highly significant pH × ejaculate interaction was found for each of the metabolic measures, except for the R.Q. values, a large part of the variance of which was associated with the individual ejaculates.

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ROLE OF BUTTERFAT IN NUTRITION AND IN ATHEROSCLEROSIS: A REVIEW

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The role of butterfat in nutrition and in atherosclerosis is discussed in the light of its chemical and nutritional properties, its importance in health, and the transitory evidence for the dietary linkage of fat with heart disease. The triglyceride and fatty acid composition of butterfat is structurally different from other edible fats. This difference contributes to the superior nutritional value of thermally oxidized butterfat. It is emphasized that the total caloric intake and the ratio of dietary protein to dietary fat may be more important in determining serum cholesterol levels than the intake of the so-called soft and hard fats. *Editor.*

Comparative growth studies under standardized conditions have served as a classical means of measuring the nutritive value of fats *(17)*. However, since high-fat diets have been used as the most successful means for inducing atherosclerosis in animals *(31)*, another criterion for judging the nutritive value of fats seems to have become available. In fact, a decrease in the consumption of dietary calories from fat, and the substitution of "soft" fats, such as corn oil, for "hard" fats such as butterfat, have been suggested as the best means for preventing atherosclerosis and coronary heart disease *(33)*.

In order to evaluate the role of butterfat in nutrition, and its role in atherosclerosis, three major factors will be considered in the present discussion: *(1)* a comparison of the chemical and nutritional characteristics of butterfat with those of other fats, *(2)* the vital role that dietary fats play in good nutrition and human health, and *(3)* the incomplete data on which practical dietary information is linked with heart disease.

CHEMICAL COMPOSITION OF BUTTERFAT AS COMPARED WITH OTHER FATS

The term "fat" usually has been applied to a wide variety of fat-soluble, or "lipid," materials. However, the component of greatest nutritional interest in this discussion involves the portion known chemically as a triglyceride. A triglyceride contains glycerol and three parts, or moles, of fatty acids.

The proportion of various triglycerides and the mixed fatty acid composition of an edible fat determine its physical state and stability and, as has been shown recently *(28)*, are important factors in its nutritional value. A fat with a large proportion of trisaturated glycerides (Table 1), as beef fat, hydrogenated coconut oil, and butterfat, is a solid or hard fat at room temperature, and is more stable towards heat than an oil or soft fat, such as safflower or corn oil, which contains a large proportion of triunsaturated or diunsaturated glycerides.

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TABLE 1

Comparison of triglyceride composition of milk fats with other edible fats
(mol. %)

Fat	GS_3	GS_2U	GSU_2	GU_3
	(%)			
Cottonseed oil	0	13	59	28
Corn oil	0	2	40	58
Palm oil	9	22	62	7
Olive oil	0	0	45	55
Coconut oil	84	12	4	0
Human milk fat	9	40	43	8
Butterfat	28	31	39	2
Lard	2	26	55	17
Beef fat	15	46	37	2

GS_3 — trisaturated glycerides; GS_2U — disaturated glycerides; GSU_2 — monosaturated glycerides; GU_3 — triunsaturated glycerides.

Curiously enough, a hard fat, such as butterfat, can contain approximately 10% less long-chain fatty acids than a soft fat, such as palm oil (Table 2). Soft fats, such as safflower, cottonseed, and corn oil contain only from 10 to 20% less long-chain saturated fatty acids than butterfat. However, these vegetable oils differ markedly from butterfat in linoleic acid content. They contain approximately ten times more linoleic acid than butterfat, and are liquid at room temperature, because they contain a larger proportion of triunsaturated glycerides than butterfat. Although differences of opinion have been expressed in regard to the nature of the linoleic acid in butterfat *(24)*, the latter has been used successfully as a biological source of essential fatty acids in fat-deficient rats *(42)*.

The small amount of triunsaturated glycerides in butterfat seems to differ from the triunsaturated glycerides of other edible fats. This difference can be demonstrated best by fractional crystallization of butterfat from acetone *(9)*. The characteristics of the various fractions obtained by low-temperature fractional crystallization from acetone indicated that the highly unsaturated linoleic acid is not concentrated excessively in any one fraction (Table 3). The Reichert-Meissl value indicated that butyric acid served in the same role as an unsatu-

TABLE 2

Comparison of total saturated and unsaturated fatty acids, in soft and hard fats

Source of fat	Saturated		Unsaturated	
	$-C_{16}$	$C_{16}+_{18}$	Oleic	Linoleic
Soft fat	(%)			
Safflower oil	1	12	20	67
Cottonseed oil	1	23	29	45
Corn oil	1	12	45	42
Peanut oil	1	18	59	22
Palm oil	2	46	43	9
Olive oil	10	3	80	7
Coconut oil	80	11	6	2
Hard fat				
Human body fat	4	32	52	9
Human milk fat	17	35	37	8
Butterfat	20	37	36	5
Lard	1	41	49	6
Beef fat	3	49	42	2

($-C_{16}$) — less than palmitic acid in chain length; (C_{16}) — palmitic, (C_{18}) — stearic acid.

TABLE 3

Characteristics of triglycerides obtained by fractional crystallization of butterfat from acetone

Temp. of crystallization	Wt. of fraction	Reichert-Meissl value	Tri-saturated glycerides	Oleic Acid	Linoleic Acid	Saturated acids
(°C.)	*(%)*					
20	9.2	4.2	86.1	10.0	0.2	89.9
10	4.9	5.6	70.5	16.0	0.3	83.7
5	7.0	19.2	68.0	17.7	0.5	81.8
0	15.0	30.2	64.4	17.9	0.7	81.4
− 5	11.4	27.9	63.6	20.9	0.8	78.3
−10	2.6	27.0	60.9	21.6	0.9	77.5
−15	4.4	26.8	48.3	30.1	1.1	68.9
−20	3.4	26.9	42.8	33.2	1.2	65.6
−25	11.0	26.9	37.2	44.1	1.3	54.2
−30	8.4	29.9	35.6	44.5	1.3	54.4
−35	2.5	—	25.2	49.7	1.5	49.6
−40	2.2	45.2	22.5	50.4	2.0	47.5
−45	5.0	47.9	18.8	51.0	2.5	46.1
−55	5.0	50.3	18.9	50.0	2.8	46.8
−65	4.0	56.7	16.2	51.4	4.1	43.9
Filtrate	4.0	64.0	8.4	63.5	6.3	29.2

rated fatty acid, and was concentrated in the most acetone-soluble triglyceride fraction. In other edible fats this fraction is usually highly unsaturated *(6)*.

One of the monounsaturated fatty acids in butterfat, *trans*-△ 9-octadecenoic or vaccenic acid, was at one time believed to possess superior nutritive properties *(11)*. The presence of 4 to 11% of various *trans* fatty acids has also been reported in cattle and sheep depot fats *(23)*. Although *trans* fatty acids do not seem to be normally present in nonruminants, they have been found in the depot fats of rats which had been fed *trans* fatty acids *(17)*, and they are also present in human tissue *(29)*. These *trans* fatty acids do not need to originate in ruminant fats, as considerable amounts of *trans* fatty acids are formed during the selective hydrogenation of vegetable oils *(3)*. Up to 42% has been reported to be present in margarine fats *(38)*. The 4 to 5% of *trans* fatty acids in butterfat thus would have no nutritional advantage over those in margarine fat.

Aroma and flavor components. The butter, as well as the oil and fat, industry has used the organoleptic, or flavor-scoring, technique for quality control. Oils and fats with an unacceptable flavor are either reprocessed or sold as grease and soap stock, and are not sold for edible purposes. In their preparation for edible use, vegetable oils normally are subjected to a more drastic processing procedure than butterfat. Vegetable oils are bleached, deodorized, and hydrogenated at relatively high temperatures *(5)*. Butterfat, on the other hand, is obtained by the simple mechanical process of churning *(6)*. Many studies have been made on the comparative growth-promoting value of hydrogenated *vs.* animal fats, and no apparent differences in nutritional value have been noted *(17)*. However, information is still lacking on the effects of selective hydrogenation and excessive heat treatment on the long-term physiological properties of fats processed, or treated, in this manner.

Recently, a method has been developed to test for the presence of carbonyl, or flavor-producing, compounds in an edible fat. The results correlate with the

organoleptic evaluation of the fat in question *(14)*. The organoleptic characteristics of the carbonyl compounds of autoxidized fats should not be confused with the desirable carbonyl compounds, such as the diacetyl in fresh milk fat. Some of the desirable carbonyl compounds are, no doubt, still present in butterfat, even after years of cold storage. However, it would be difficult to distinguish them from the carbonyl compounds that are formed by autoxidation. They can be isolated by a process in which the phenylhydrazone derivatives are characterized by microanalytical techniques *(12, 13)*. The presence of both desirable and undesirable carbonyl compounds has added to the difficulties involved in studies on oxidized flavors in milk and other dairy products *(32, 43)*.

The aroma and flavor components associated with fats are among the chief organoleptic factors contributing to dietary patterns. Although it would be more economical to eat a balanced poultry ration, which would be low in fat, high in vitamins and protein, and complete in all of the essential dietary components, such a diet is not palatable to human beings. Palatability, food availability, previous dietary habits, and emotional factors, rather than good nutrition may, therefore, play a major role in establishing individual dietary patterns.

It is virtually impossible to cook a palatable meal, or to bake, without fat. In baking, fats lubricate and weaken the structure of wheat gluten to shorten and tenderize the product *(5)*. The recipes which have eliminated fat from the diet *(18)* are often abandoned, because they fail to recognize that aroma and flavor are more important to the human dietary than fat percentages and fat calories.

Nutritive value of heated butterfat as compared to heated corn oil. To study the comparative nutritive value of butter and vegetable fats, we took advantage of the stress which is placed on animals fed fats damaged by heat *(27, 28)*. Three different types of heated or polymerized fats were used which, for the sake of convenience, were named "oxidatively," "thermally," and "oxidative thermally" polymerized fats. The results indicated that the rate of growth on oxidatively or thermally polymerized fats did not vary significantly from rate of growth on the fresh fat (Table 4). However, the rate of growth on oxidative thermally polymerized corn oil was drastically depressed, when compared with growth on butterfat that had been treated in a similar manner *(26)*. Thus, prolonged heat treat-

TABLE 4

Comparison of butter oil and corn oil fed to rats

Fat	Polymerization	Weight at:	
		Beginning	8 wk.
Butter	None	58	281
Butter	Oxidative	59	293
Butter	Thermal	56	294
Butter	Oxidative-thermal	58	280
Corn oil	None	51	309
Corn oil	Oxidative	57	307
Corn oil	Thermal	59	304
Corn oil	Oxidative-thermal	59	133

ment in the presence of air may harm the nutritive value of fats containing a substantial quantity of triunsaturated glycerides, such as corn, cottonseed, olive, and soybean oils.

THE VITAL ROLE OF DIETARY FATS

Dietary fat is not only necessary as a nutritional component but as a component vital to life and to the structure of the cell itself. Fat is present in muscle tissue as visible fat, and as invisible fat in the actin and myosin, which are vital protein components of the individual muscle cells *(10)*. This fat is very complex, and is combined with protein in a manner still largely unknown. It cannot be removed from the living cell without fatal injury to it. Dietary fat is also essential to many physiological processes in the body. It has a protein-sparing value, is needed for the optimum utilization of lactose, and serves as a carrier for fat-soluble vitamins *(17)*. Dietary fat is beneficial in stress situations, such as injury due to radiation or excessive exposure to cold. The fat deposited around vital organs, such as the kidneys, serves to protect them against mechanical injury, and the fat deposited under the skin serves as an effective heat-insulator.

Absorption, digestion, and body composition. The absorption of a fat is measured in terms of rate. Digestibility refers to the percentage of fat absorbed. The rate of absorption of edible fats is in the same range, although butterfat seems to be absorbed more readily than other fats *(17)*. All animal and vegetable fats which melt below 50° C. are completely digested by the normal individual. Therefore, one fat is not more digestible than another. The stomach is important to fat digestion, as it sends the chyme into the intestine at a rate at which the body can use it. Therefore, the effect of fat on slowing the emptying-rate of the stomach contributes to the satiety value of food *(8)*.

The fat absorbed through the lymph and blood either is deposited in the various tissues or is used immediately as a source of energy. Normal human blood plasma contains from 245 to 470 mg. fatty acids, 120 to 350 mg. phospholipids, and 110 to 310 mg. cholesterol per 100 ml. of plasma *(10)*. A portion of the dietary fat which enters the blood via the lymph may first reach tissue other than the liver, and may be deposited in the adipose tissue in the form of depot fats. Nevertheless, the liver appears to represent a major site of fat transformation.

The fats are in a continuous state of flux, with the fatty acids moving from the fat depots to the liver, and vice versa *(20)*. Therefore, even though a wide variety of fats is consumed, the mixed fatty acid composition of the body fat is fairly characteristic of the species *(24)*. As the human diet contains a wide variety of foods, the body cells can pick out the essential fatty acids they need for their own maintenance and metabolize or burn up the remainder as a source of body heat and energy *(7)*.

The human body contains a total of approximately 20% of ether-extractable, or lipid material *(19)*. The amount of lipid varies with different tissue. Adipose tissue contains 72% lipid; heart, 17; skin, 14; striated muscle, 7; liver, 3, and

lungs, 1%. The adipose tissue fat *(16)* contains the saturated fatty acids myristic, palmitic, and stearic acid, and the unsaturated oleic acid in the proportions shown (Table 2). These fatty acids may be classified as the nonessential fatty acids, since all of them can be synthesized in animal tissue from nonfat precursors. In addition, human body fat contains approximately 9% linoleic and 1% arachidonic acid, which contain two and four double bonds, respectively *(16)*. These two fatty acids have been classified as the essential fatty acids because linoleic acid does not seem to be synthesized in animal tissue but serves as the essential precursor for the synthesis of arachidonic acid.

The 1% of arachidonic acid *(16)* in human fat is either synthesized from a dietary source of linoleic acid, or consumed as a component of animal fats, such as butterfat. The fat present in vegetables, fruits, and cereals contains as high a proportion, although not as large an amount, of linoleic acid as corn oil; and eggs, meat, and butterfat all contain the essential arachidonic acid. Vegetable oils or hydrogenated vegetable oils do not contain arachidonic acid. However, when an adequate amount of pyridoxine is fed with a source of linoleic acid, arachidonic acid is synthesized in the body. The heart, liver, brain, and other vital organs contain higher concentrations of linoleic and arachidonic acid than the adipose, skin, and skeletal muscle tissue *(44)*.

The "essential" fatty acids. The 9% of essential linoleic acid, which was shown to be present in human fat *(16)*, supposedly is all consumed as a component of dietary fat. Most of the experimental proof for the need of dietary sources of essential fatty acids has been obtained with the rat as the experimental animal, for it would be difficult to feed a fat-deficient diet to man long enough to produce essential fatty acid–deficiency symptoms. However, human infants on a fat-free diet have developed an eczema, which was accompanied by a lowering in serum linoleic and arachidonic acid values *(22)*. On the basis of this evidence, and on the observation that rats kept on a fat-deficient diet develop a characteristic dryness and scaliness on the hind feet and tail, the essential fatty acids are believed to be necessary for the maintenance of normal skin tissue *(25)*.

Female rats which had been raised to maturity on a diet deficient in fat, but adequately supplied with other dietary factors and bred with normal males, could not give birth to normal living young unless unsaturated fat was supplied in the diet *(36)*. Total fat analyses of representative female rats and their newly born young indicated that the fat-deficient animals, that is, the animals which been been kept on the fat-free diet, were deficient in arachidonic acid and not deficient in fat as such. It is, therefore, evident that dietary fat is essential for normal reproduction and lactation, to supply the essential fatty acids which cannot be synthesized from carbohydrate and protein.

DIETARY INFORMATION AS LINKED WITH HEART DISEASE

Human atherosclerosis, the most prevalent type of arteriosclerosis, is considered responsible for 25% of all deaths in the United States *(39)*. Clinical observations have shown that a high correlation exists between obesity and atherosclerosis *(15)*. Clinical tests, such as cholesterol and lipoprotein, or S_f

10-20 molecule determinations of blood serum *(21)*, also seem to indicate a correlation between dietary fats and atherosclerosis. Furthermore, the feeding of large amounts of cholesterol to rabbits and chickens leads to the deposition of cholesterol in the arterial walls, and to atherosclerosis. Therefore, attempts *(30, 33, 37)* to establish a relationship between human atherosclerosis and the ingestion of fats which contain cholesterol, such as butterfat, may have a seemingly logical basis. However, the total caloric intake may be more important to cholesterol synthesis than a low-fat, low-cholesterol diet. Butterfat contains approximately only 0.8 g. of cholesterol per lb., whereas man has been estimated to synthesize more than 15 times this amount of cholesterol daily in his own tissue. The in vivo synthesis of either fat or cholesterol would not be restricted by consuming a low-fat, low-cholesterol diet, unless the caloric intake would be curtailed drastically enough to interfere with normal fat and cholesterol synthesis.

Current advice on low-fat diets as related to atherosclerosis often rests on incomplete knowledge in regard to the effect of one dietary component on another. In human subjects on inadequate diets *(33)*, such as the Bantu, a deficiency in caloric intake, rather than the level of dietary fat, may be the limiting factor in atherosclerosis. In American diets, the total caloric intake is excessive; under these circumstances, the ratio of dietary fat to protein may be the limiting factor.

The assumption that metabolic adjustments can be made, no matter how much fat or carbohydrate is eaten, can be more easily studied with chickens than with human subjects. We found a direct relationship between dietary carbohydrate, fat and protein intake, and carcass composition *(41)*. For example, more fat was found in the tissue of birds which had been kept on a low-protein than on a high-protein diet. The level of dietary fat did not influence the total fat composition of the body, if an adequate amount of protein was consumed. It is entirely possible that human subjects are as variable in body composition as chickens, and that this variability may have a significant relationship to faulty metabolism and atherosclerosis.

In experimental studies involving shifts in serum cholesterol, it has been assumed that a decrease in serum cholesterol is desirable *(2, 30, 33)*. However, until a complete cholesterol balance can be obtained in diet experiments, such assumptions may not be valid. A decrease in serum cholesterol may also be due to an increase in the deposition of the cholesterol in the liver or carcass *(1)*. In fact, until complete balance studies on total as well as serum cholesterol are available, observations of changes in serum cholesterol when lecithin, sitosterol, and other agents are administered, should be regarded with caution.

We have found that a high-fat diet increased serum cholesterol values significantly, but only when the level of dietary protein was inadequate *(35)*. A decrease in serum cholesterol may occur by rigidly eliminating fat from the dietary regimen, as this practice may unintentionally increase the protein intake through the substitution of high-protein foods for fat. If, however, substitution is done blindly, it may result in substituting carbohydrate for fat and thus may not increase the dietary protein level. A drop in serum cholesterol may also

occur, but it may not be as rapid as when the dietary protein level is increased.

It has been suggested that the "practical" diets of subjects who have suffered a heart attack should contain unhydrogenated oils instead of butterfat or hydrogenated fats *(34)*. This recommendation has been made for a number of reasons. It has been noticed that population groups on vegetable oils have lower serum cholesterol levels than those on butterfat or hydrogenated fats, and the shifting of heart patients from butterfat to corn oil, in a diet in which more than 60% of the calories was supplied by fat, lowered serum cholesterol levels *(2)*. Furthermore, the transport of cholesterol in fat-deficient animals becomes more effective when essential fatty acids are fed *(1)*. Essential fatty acids no doubt have an important role in cholesterol transport. It is possible that the cholesterol esters formed from essential fatty acids may be cleared from the serum more easily than those formed from saturated fatty acids. However, the recommendations in regard to the effect of hard and soft fats on serum cholesterol levels were based on experimental diets excessively high in fat calories. The data should, therefore, not be extrapolated to include a normal American dietary pattern.

It may be desirable to lower the consumption of foods high in calories, such as French-fried potatoes, potato chips, and party snacks, if these items are consumed at the expense of high protein–containing foods, such as milk, eggs, and meat. Replacing fats with foods containing a large proportion of carbohydrate such as rice, candy, soft drinks, and other similar food items, seems no solution to the problem of excessive caloric consumption and cholesterol synthesis, as carbohydrates can be converted into fats and cholesterol in the body and stored in the body-fat depots. More fat may be stored, therefore, through an excessive intake of carbohydrate than through a moderate intake of fat. This does not mean that we should stop eating rice, candy, or high energy–containing foods. It does indicate that high-protein foods such as milk, meat, and eggs should serve as the foundation of our diet, even though these food items also contain animal fats. Adding fruits and vegetables will help to supply vitamins and make this diet complete in all essentials. If the energy needs are still inadequate, they can be made up by eating small amounts of high-energy foods. This approach to eating would seem to provide the dietary essentials more adequately than a haphazard consumption of high-carbohydrate foods just because they are low in fats, and would result in better nutrition than cutting out a source of animal protein, such as whole milk, simply because it contains a small percentage of hard fat.

One indirect answer has been provided to the heart disease problem through the classical dietary approach which has been used in the development of experimental atherosclerosis. Except for an imbalance in hormone metabolism, it always has been necessary deliberately to upset the balance of dietary fat to protein, in order to develop experimental atherosclerosis *(4, 31)*. This has been done most easily by adding excessive amounts of fat and cholesterol to an experimental diet, thus depressing the protein content to a deficiency level. It would, therefore, seem that when the caloric intake is more than adequate, it may be important to supply an optimum amount of lipotropic factors, to offset the cholesteremic effect of a high-fat diet.

SUMMARY

In summary, it has been shown that the triglyceride, as well as the fatty acid composition, of butterfat is different from other edible fats. This difference in triglyceride structure seems to contribute to the superior nutritional value of thermally oxidized butterfat. It has also been pointed out that the total caloric intake and the ratio of dietary protein to dietary fat may be more important to serum cholesterol levels than soft or hard fats.

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DEFECTS PRODUCED BY SPOILAGE ORGANISMS IN COTTAGE CHEESE STERILIZED BY CATHODE RAYS [1]

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Five genera of bacteria, two of yeasts and three of molds, were isolated, identified and inoculated, individually and with Streptococcus lactis, into cottage cheese which had been sterilized by cathode rays at 5.5 x 10⁶ rep. Two species of Achromobacter, one Alcaligenes, two Escherichia, four Pseudomonas, one Rhodotorula, and one Torulopsis all produced slime on cottage cheese. The characteristics of the slime varied with the species. Species of Achromobacter and Escherichia produced defects quicker when inoculated alone, but species of yeasts and three Pseudomonas cultures produced defects quicker with S. lactis. The molds produced pigmented growth characteristic of each genus. *Editor.*

Increasing interest in the keeping quality of cottage cheese has resulted in studies involving the identification of specific organisms responsible for spoilage. Elliker *(6)* identified *Pseudomonas viscosa*, *Pseudomonas fragi*, and *Alcaligenes metalcaligenes* as being associated with slime formation in cottage cheese, and described the defect caused by each organism. Davis and Babel *(4)* found that a number of species in the *Proteus*, *Pseudomonas*, *Aerobacter*, *Alcaligenes*, and *Achromobacter* genera were capable of producing a slimy defect in cottage cheese. The importance of pH in controlling spoilage has been reported *(3-6)*.

In working with defects in cottage cheese, frequently spoilage organisms were encountered belonging to various genera, including numerous yeasts and molds. Therefore, it seemed desirable to isolate and identify the species and determine the characteristic defect produced in sterilized cheese by the more common ones encountered.

EXPERIMENTAL PROCEDURE

Cottage cheese samples were collected from commercial plants and retail outlets and held at 10 and 20° C. until spoilage occurred. The organisms were isolated by plating at high dilutions and by repeated streaking on tryptone glucose yeast agar or acidified potato dextrose agar, prepared according to Standard Methods *(1)*. A total of 32 cultures was isolated and, after elimination of duplicates, 17 were selected for investigation.

The organisms are listed (Table 1). The bacteria include two species of *Achromobacter*, one of *Alcaligenes*, two of *Escherichia*, three of *Micrococci*, and four of *Pseudomonas*. Also included are one *Rhodotorula* and one *Torulopsis* yeast and one each of *Geotrichum*, *Mucor*, and *Penicillium* molds.

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TABLE 1

Defects produced by different organisms, inoculated individually and in combination with S. lactis, into sterilized cottage cheese at pH 5.10 and incubated at 20° C.

Organism	Sterilized cheese inoculated with individual cultures			Sterilized cheese inoculated with individual cultures and with *S. lactis*		
	Days to spoil	pH when spoiled	Appearance and odor	Days to spoil	pH when spoiled	Appearance and odor
Bacteria						
A. butyri	2	5.05	Clear, gelatinous slime, fermented	3	5.20	Clear, gelatinous slime, fermented
A. eurydice	2	5.05	Gray gelatinous slime, fermented	3	5.25	Gray gelatinous slime, fermented
A. metalcaligenes	4	5.35	Tapioca slime, no odor	3	5.40	Tapioca slime, no odor
E. coli	3	5.85	Brown slime, unclean	4	5.75	Brown slime, unclean
E. freundii	3	5.30	Brown slime, unclean	4	5.50	Brown slime, unclean
M. candidus	5	4.60	Whey, sour	5	4.45	Whey, sour
M. conglomeratus	5	4.60	Whey, sour	5	4.55	Whey, sour
M. flavus	4	5.20	Yellow viscid buttons, no odor	5	5.10	Slight yellow pigment, no odor
P. desmolyticum	3	5.40	Tapioca slime, fermented	2	5.40	Tapioca slime, fermented
P. fluorescens	3	5.25	Greenish brown slime, putrid	5	5.15	Greenish brown slime, putrid
P. fragi	3	5.10	Light brown slime, fruity	2	5.15	Light brown slime, fruity
P. tralucida	3	5.30	Grayish brown slime, fermented	2	5.40	Grayish brown slime, fermented
Yeasts						
R. species	4	5.40	Pink slime, yeasty	3	4.75	Pink slime, whey, yeasty
T. candida	4	5.70	Yellow slime, digested, fermented	3	5.60	Yellow slime, digested, fermented
Molds						
G. candidum	3	5.25	Leathery light brown, moldy	2	5.25	Leathery light brown, moldy
M. plumbeus	3	5.60	Gray, moldy	3	5.30	Gray, moldy
P. frequentans	3	5.40	Blue-green moldy	3	5.50	Blue-green moldy

Tests required for identification were performed as recommended by Breed *et al. (2)*, Gilman *(7)*, Lodder and Kreger van Rij *(8)*, Raper *et al. (9)*, and the Society of American Bacteriologists *(10)*. Several of the bacterial cultures exhibited one or more minor variations from the characteristics listed by Breed *et al. (2)*, but were tentatively assigned to the species most accurately coinciding with their biological activities. Except for the *Rhodotorula* yeast, the characteristics of the mold and yeast cultures conformed to authoritative descriptions of the species *(7, 8, 9)*.

Cottage cheese contains substantial numbers of lactic organisms, mostly *Streptococcus lactis*, introduced as a culture in the manufacturing process, along with varying numbers of contaminants introduced during processing and packaging. Previous work *(3, 4, 6)*, involving description of defects caused by certain spoilage organisms, has involved the inoculation of the organisms into normal cottage cheese. In this work, normal commercial cottage cheese with a pH of 5.1 was sterilized prior to inoculation, using cathode rays. By experimentation it was established that 15 to 16 g. of small-curd cottage cheese, pressed into a layer not exceeding $\frac{1}{8}$-in. thick, in a standard 85 mm. i.d. petri dish, could be sterilized by an exposure to cathode rays at a dosage of 5.5×10^6 rep (Roentgen equivalent physical). During sterilization, each of the bottom sections of the petri dishes containing the cheese was covered with a thin sheet of polyvinyl chloride–vinylidine chloride (Saran wrap), which was subsequently replaced with a sterile petri dish cover.

Uninoculated control plates sterilized in this manner were held for 30 days at room temperature without evidence of spoilage, and no organisms were recovered by plating procedures. Cheese sterilized using cathode rays was darkened in color and possessed an objectionable flavor.

Each of the dishes of sterilized cheese was inoculated with 0.1 ml. of an 18-hr. trypticase soy broth culture of the spoilage organism. A duplicate set of plates received a similar inoculation of *S. lactis*, in addition to the contaminant. *S. lactis* was introduced because it represents the majority of the normal bacteriological populations of cottage cheese. The plates were incubated at 20° C. until spoilage occurred, which required from two to five days.

RESULTS AND DISCUSSION

The length of time required for spoilage to occur, description of the spoilage, and pH of the spoiled cheese in each of the inoculated dishes are shown (data in Table 1).

Pure cultures of the species of *Achromobacter* and *Escherichia* caused spoilage quicker than the same cultures mixed with *S. lactis;* however, the difference in time was insufficient to warrant assigning any significant degree of inhibitory action to *S. lactis*. In most instances, the species of *Pseudomonas* and the yeasts produced spoilage more rapidly when combined with *S. lactis* than when in pure culture. The presence of *S. lactis* did not seem to influence the time required for the *Micrococci* and the molds to induce spoilage.

Most of the organisms caused an increase in pH varying from slight to substantial, when inoculated both alone and in combination with *S. lactis.* These results agree with those of other workers *(3, 4, 6),* who studied slime formation by species of *Pseudomonas* and *Alcaligenes.* Two species of *Micrococci* and the *Rhodotorula* yeast combined with *S. lactis* caused marked decreases in pH, sour aroma, and liberation of whey.

It was anticipated that there might be some difference in the appearance and type of spoilage produced by pure cultures, as compared to the same cultures mixed with *S. lactis;* however, in general, the discoloration and odor were approximately the same, regardless of whether *S. lactis* was included. It is probable that the pH decreased too rapidly for pigment production in the samples inoculated with *Micrococcus candidus* and *Micrococcus conglomeratus,* which usually produce white and yellow buttons, respectively, on standard agar *(2).* There was no substantial difference in the spoilage caused by these organisms in unsterilized cottage cheese, as compared to sterilized cottage cheese.

It is noteworthy that most of the samples showed a substantial increase in pH when spoiled, and that spoilage was generally more rapid in the samples in which the pH increased. There were a few exceptions, such as samples inoculated with *M. candidus* and *M. conglomeratus,* in which acid production was a contributing cause of spoilage, and deterioration occurred in low pH samples.

SUMMARY AND CONCLUSIONS

Seventeen organisms representing five genera of bacteria, two genera of yeast, and three genera of mold were inoculated individually and in combination with *Streptococcus lactis,* into cottage cheese which had been sterilized by cathode rays at a dosage of 5.5×10^6 rep.

Achromobacter butyri and *Achromobacter eurydice* produced gelatinous slime and a fermented aroma. *Alcaligenes metalcaligenes* and *Pseudomonas desmolyticum* produced a tapioca slime, with the former causing no odor and the latter causing a fermented aroma. Species of *Escherichia* and *Pseudomonas,* and the *Rhodotorula* and *Torulopsis* yeasts, all produced surface slime. The aroma, pigment, and physical nature of the slime varied with the species. *Geotrichum candidum, Mucor plumbeus,* and *Penicillium frequentans* all produced pigmented mold surface-growth, characteristic of the particular species.

Cultures of species of *Achromobacter* and *Escherichia* produced defects more rapidly when inoculated individually than when combined with *S. lactis.* Cultures of species of yeast and three of the *Pseudomonas* cultures produced defects more rapidly when combined with *S. lactis* than when inoculated alone. The appearance and odor of the spoiled cheese was the same, regardless of whether the cultures were introduced individually or in combination with *S. lactis.*

Most of the organisms increased the pH during the period required for spoilage, notable exceptions being two species of *Micrococci* and the *Rhodotorula* yeast combined with *S. lactis,* all of which caused substantial production of acid and whey.

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TECHNICAL NOTES

I^{131} UPTAKE IN CALVES FED CHLORTETRACYCLINE

The growth-stimulating effects of chlortetracycline and other antibiotics in dairy calves have been reviewed recently by Lassiter *(2)*, who discussed various theories of their mode of action. Calesnick *et al. (1)* reported that both chlortetracycline and penicillin, fed at the rate of 1 mg/kg of feed to two groups of four rats, had a goitrogenic effect as measured by increased thyroid weight and decreased I^{131} uptake. The purpose of this experiment was to determine whether or not growth stimulation in calves resulting from chlortetracycline might be associated with thyroid function.

In this study, I^{131} uptake was measured in calves which had been fed chlortetracycline to 12 weeks of age.

In Experiment I (1953), 16 Jersey calves were used, nine of which were fed 20 mg. of chlortetracycline (Aurofac-2A) per lb. of dry feed. The dry ration consisted of high-roughage pellets (two parts ground alfalfa hay and one part a simple grain mixture). Whole milk was fed to seven weeks of age and free access to iodized salt was provided. Seven similar calves did not receive chlortetracycline, and served as controls.

Near the end of the 12th week, the calves were injected intraperitoneally with either 20 or 80μc. of I^{131} in physiological saline solution. I^{131} uptake was measured as counts/min in vivo 24 hr. after injection, using a portable rate-meter equipped with a Geiger Mueller tube. The averages of three readings were used. These were taken while holding the calf with nose raised and head turned slightly to the side. The probe was held in contact with the skin over the thyroid gland and manipulated until the area of maximum reading was located. The results of the I^{131} uptake measurements (counts/min/μc/100 lb. body wt.), gain in weight to 12 weeks of age, and average chlortetracycline intake/day at 12 weeks are shown (Table 1). Although growth was increased by chlortetracycline feeding, no differences in I^{131} uptake were observed.

In Experiment II (1954), 10 Jersey calves were used, five of which were fed chlortetracycline (100 mg/day mixed in warm water). During the 12th week, each calf was injected intraperitoneally with 100 μc. of I^{131} in physiological saline, and the iodine uptake determined in vivo 24 hr. later. These results are also shown (Table 1). As in Experiment I, no effect of chlortetracycline on I^{131} uptake was noted.

The difference between the average values obtained in the two experiments is thought to be due principally to the different instruments used,[1] and the possible difference in iodine intake, which was not regulated. However, the inclusion of chlortetracycline in the feed did not alter thyroid activity as measured by I^{131} uptake in either experiment. These findings are in agreement with the results of Telkka and Kuusisto *(4)* using rats, and of Libby and Meites *(3)* using both rats and chicks, who were unable to demonstrate any goitrogenic effect of chlortetracycline.

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[1] Experiment I—Thyac Survey Meter, Model 389, and E1-Tronic Beta-Gamma Survey Meter, Model P.R.-3. Experiment II—Nuclear-Chicago Rate Meter, Model 1615-B, equipped with Model P 11 probe, containing a model D 33 end-window counter (mica 3.5 mg/cm^2).

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TABLE 1

Effect of chlortetracycline on average weight gain and I^{131} uptake in Jersey calves 12 weeks of age

Group	Calves	Daily chlortetracycline intake at 12 wk.	Weight gain to 12 wk.	μc/100 lb.
	(No.)	*(mg.)*	*(lb.)*	*(c/min.)*
Experiment I				
Experimental	9	110.8	85.0[a]	31.8 ± 9.7
Control	7	0.0	69.9	32.2 ± 12.7
Experiment II				
Experimental	5	100.0	82.4	18.2 ± 10.5
Control	5	0.0	77.2	18.2 ± 9.3

[a] Mean differences in body weight gains significant at the 5% level of probability.

RENNET ACTION AND EXCHANGEABILITY OF CALCIUM IN MILK TO ION EXCHANGE RESINS

The exchangeability of calcium to ion exchange resins is known to be a specific property of milk, and variations in this, resulting from heat treatment and cool-aging, are measurable. These can be studied by the Resin-Contact-Time-Method *(2)*. Thus, Baker and Gehrke *(1)* reported the influence of heat treatment on the exchangeable calcium in milk. Gehrke and Smith *(3)* have further studied the effects of heat and pH on the exchangeability of calcium and magnesium in milk. Rennet is known to influence the base-binding capacity of the casein *(4)*, and it was thought of interest to see if rennet would cause any alteration in the nature and amount of exchangeable calcium in milk.

Using a buffered resin mixture consisting of 2.5 g. of K-form and 3.5 g. of H-form, of Amberlite IRC-50 in the dry state, which gave a pH of 6.8 on soaking in water overnight, the exchangeability of calcium was studied on fresh skimmilk, with and without added rennet. The rennet was allowed to react at room temperature and at 8° C., to see whether the difference, if any, was owing primarily to the enzymic phase of rennet action. After the rennet was allowed to react for an appropriate time, 25 ml. of the sample was contacted with the resin for 20 sec. and separated from the resin mixture by filtering in a sintered-glass crucible over vacuum. The effluent collected was analyzed for calcium. The exchangeable calcium was computed by subtracting the amount in this effluent from the total calcium content of the milk sample.

TABLE 1

Exchangeable calcium as per cent of total calcium in milk, determined by Resin-Contact-Time-Method

Skimmilk control	Skimmilk + rennet			
	About 20° C.	Time[a]	8° C.	Time[a]
(%)	*(%)*	*(min.)*	*(%)*	*(min.)*
55.9	55.9	5	51.0	30
53.5	55.5	5	59.4	30
55.0	57.4	10	55.1	37
50.9	56.5	10	52.5	40
54.3	53.5	10	54.2	75
55.1	53.5	20	55.9	75
	55.0	20	50.6	80
	56.5	20	53.5	85
			52.3	90
Av. 54.1	55.5		53.8	

[a] Minutes that milk contacted rennet.

Results are presented in Table 1, which gives the exchangeable calcium as per cent of the total in milk, and the enzyme reaction time in minutes. The averages for exchangeable calcium include the replicates, with varying rennet contact times, both at room temperature and at 8° C. The exchangeable calcium in the skimmilk control was 54.1%, as compared with 55.5 and 53.8% for rennet-treated milk at room temperature and at 8° C., respectively. These variations do not reflect a significant difference in the exchangeability of calcium as a result of rennet action, and these results do not confirm our initial observations *(5)*. The present results are in harmony with the observations made on the nature of casein sediment in the centrifugation studies, in which it was found that the calcium composition of the casein complex, separated by centrifugation, does not show any variations as a result of rennet action *(6)*.

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EFFECT OF TRANSPORTATION UPON FERTILITY OF BULLS

This laboratory *(3)* has previously reported data on fertility of 36 bulls, prior to and following transportation among studs, in a large artificial breeding organization. On the basis of fertility data and experience of that organization, there has been no evidence of reduced fertility caused by transportation. This has been contrary to the findings of Meschaks *(2)* and Knudsen *(1)*, who have described several bulls as incurring degenerative changes in the spermatogenic epithelium, increased neutral steroids in urine, increased morphological abnormalities of spermatozoa, and reduced fertility, following transportation. They attributed these disturbances to direct or indirect psychic effect (transport stress) on the sexual functions.

This Technical Note reports additional fertility data which the writer has accumulated since the first paper. In the present study, 60- to 90-day nonreturn rates from inseminations performed the first day following collection of semen were compiled for each of the two one-month periods preceding and following each move. The bulls included in this study were transported for distances ranging from approximately 300 to 2,000 miles. In most cases, the longer distances were by train and the shorter by truck. In one or two instances bulls were transported the longer distances by airplane. The scrota were not protected by suspensory supports. In this second study, 65 moves by 60 mature bulls, among six studs, are represented. There were four Aberdeen Angus, three Brown Swiss, 14 Guernsey, 20 Holstein-Friesian, 16 Jersey, and three Shorthorn bulls.

The results are presented (Table 1). Although there was a slight decline in nonreturn rate following moving, the means for each of the two months following moving were greater than for the second month preceding transportation. None of these differences is significant. Also, the average nonreturn rates for the two months preceding, and for the two months following, moving were both 70.2%. There was, however, a significant ($P < 0.05$) interaction between bulls, on the one hand, and nonreturn rates before and after moving, on the other. When comparing nonreturn rates of individual bulls before and after moving, marked increases and decreases in nonreturn rates were found. For example, the extremes were 13 percentage units, with two bulls having an increase and one a decrease of this magnitude. Marked differences in both directions were explained by differences in management, fertility levels of studs, and by chance. Where bulls had extremely low nonreturn rates following moving, there was no evidence that this condition was permanent.

TABLE 1

Nonreturn rates for each of the two months preceding and following transportation of individual bulls

Period	Services	Nonreturns
	(No.)	*(%)*
2nd month before	28,413	69.6
1st month before	22,939	70.7
1st month after	29,703	70.3
2nd month after	33.787	70.0

These data support the observations in the first study. The continued experience of this artificial breeding organization is that no bull has experienced reduced fertility that could be attributed to transportation. All of these bulls not only have been carefully observed by well-trained and experienced men in the studs but also have been under close supervision by veterinarians.

Meschaks *(2)* and Knudsen *(1)* made their observations mostly with bulls of the Swedish Red and White Breed, although Meschaks reported reduced spermatogenesis following transportation of a Friesian bull. Knudsen has suggested that the Swedish Red and White Breed might have a weaker endocrine constitution, in relation to sexual functions, than do other breeds.

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SYMPOSIUM

Dr. C. F. Huffman, in the September Journal, gave the membership of the Association an excellent summary of the activities that are supported by the American Dairy Science Association. He specifically mentioned the large number of committees that are functioning in the applied and the fundamental areas of Dairy Science. The activities of these committees have extended far beyond the call of duty and the results achieved have had far-reaching effects. The Association is greatly indebted to these faithful committees for their fine contributions.

It is the policy of the Journal Management Committee to have the Editor-in-Chief keep the membership informed of the activities of these committees, by encouraging the publication of progress and finished reports when they appear timely. Such reports will be published in the Journal in the future.

The Education Committee[1], consisting of R. F. Holland, H. L. Mitten, D. L. Murray, G. H. Wise, I. W. Rupel, and E. L. Jack, Chairman, organized the symposium for the 52nd Annual Meeting at Oklahoma State University on the subject: Curriculum and Course Organization for Dairy Science. Two outstanding educators were invited to represent the Manufacturing and Production sections of Dairy Science. Their papers were stimulating. They challenged present-day concepts of educating young men for careers in the dairy industry. The discussions that followed were spirited and lively.

It is evident that most curricula in Dairy Science have lagged far behind the frontiers that our scientists have opened up for us. The addresses of the two speakers in the symposium are published in the hope that departments may evaluate curricula in the light of their remarks, and then plan educational programs that will take students to the outermost frontiers of learning and enable them to participate in exciting educational adventures, comparable to those experienced by our scientists when they probe for new facts. It is believed that this is the general thought-provoking approach to learning—the real objective of all education. *Editor.*

[1] Members of committees are published each year in the January Journal.

CURRICULUM AND COURSE ORGANIZATION FOR DAIRY SCIENCE. THE DAIRY PRODUCTION CURRICULUM

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In discussing curricula, it is difficult to provide conclusive evidence that any system of training is superior. Limitations in methods of evaluating pedagogical concepts account for the diverse programs in our secondary schools. Admittedly, these viewpoints are dogmatic.

The curriculum being submitted has much in common with one which the dairy and livestock majors at the University of California have taken for many years. Though it is one of the most demanding of the various agricultural curricula, it has also been one of the most popular. It is tempting to use this criterion for its support, but a multitude of factors are involved in determining the popularity of a curriculum. The excellence of the teaching staff is an extremely important factor. It is doubtful, however, that excellent teachers can save a curriculum in which the scope of training is too broad to allow a fundamental background in any area.

In discussing professional curricula, one must consider the objectives for prescribing any specific one. The objectives for professional curricula, in general, were well stated by a special committee of the University of California *(1)*, appointed to study how to appraise professional and vocational education:

(1) To provide the student with the necessary professional training, to successfully make a beginning in his chosen profession

(2) To provide him with a basic understanding of the place of his profession in, and its relation to, society

(3) To prepare the student for intellectual, political, and cultural citizenship and leadership

(4) To instill in the student a respect and desire for knowledge and truth which shall continue throughout his lifetime.

No doubt, if these objectives are interpreted broadly, they may be sufficiently comprehensive, but for purposes of emphasis one more could be added; namely, to train the student to evaluate the basis for specific conclusions,

whether these conclusions concern science, politics, or religion. The writer feels strongly on this point, because it is believed that a glaring defect in our society is the inability of people to detect the difference between a dogmatic conclusion and one which is arrived at on the basis of scientific facts. We see this frequently in the livestock industry, as evidenced by the inability of producers to distinguish between results of a well-controlled and well-analyzed experiment and a field trial where conditions are so poorly controlled that it is impossible to determine the significance of the results.

In prescribing a curriculum to reach the stated objectives, it would seem essential to do the following:

(1) Give sufficient sciences in order that students can comprehend biological processes

(2) Give detailed information on the management of dairy cattle, using management in the broad sense, to include not only knowledge of the genetics, physiology, and nutrition of the animal but also the producing of feeds for the animal, and marketing

(3) Provide sufficient cultural courses to prepare the students for intellectual, political, and cultural citizenship and leadership.

Dairy Production Curriculum (Minimal Requirements)

Subject	Units *
Physical Sciences	
Beginning chemistry †	10
Organic chemistry	3
Physics **†	6
Soils	3
Biological Sciences	
Biochemistry	3
Zoology †	8
Bacteriology	4
Botany	5
Animal nutrition	3
Animal physiology	5
Genetics	4
Dairy Production	
Beginning livestock husbandry (including judging)	5
Beginning dairy products	3
Feeds and feeding (including basic principles and specialized dairy nutrition)	5
Advanced dairy production (animal breeding, reproductive physiology, judging, and management)	7
Electives in dairy production, or supporting fields (e.g., agronomy, business administration, marketing, agricultural engineering)	10
Animal pathology and disease control	3
Dairy production experience ‡ (highly recommended but not required)	no credit
Miscellaneous Requirements	
English (including 3 units of English composition)	6
American history	6
Military science	8
Economics	3
Statistics	3
Electives in cultural courses	11
	124

* Number of lectures weekly per semester, or equivalent in laboratory.

** Requiring trigonometry as prerequisite.

† Same courses as recommended for medicine.

‡ Equivalent of one year of practical experience in dairying.

Proposed Curriculum

You have before you a tentative curriculum in dairy production which the writer prepared. Realizing that the allotment of subject matter between courses will vary considerably among institutions, specific courses are not cited. This curriculum is very similar to one prepared by a special subcommittee of this Association *(2)*, reported at the annual meeting in 1953. The required and recommended science courses are very similar to those recorded here, but in a comment the over-all curriculum committee states, "The basic science requirements seem high for the nontechnical curriculum. These might be modified by the elimination from that curriculum of algebra, physics, and botany, and changing the credits for several courses as follows: general chemistry, 6; general bacteriology, 3; animal physiology, 4, and genetics, 3." In other words, the over-all curriculum committee in this brief statement completely emasculated the subcommittee report, from the standpoint of scientific background. How could one expect the biological science courses to be taught adequately to students with such a fragmentary background? To be precise, the excellent report made by the subcommittee for the production curriculum was nullified by the over-all curriculum committee.

It may seem that the proposed requirements in physical and biological sciences are excessive, but these, in the writer's opinion, are essential if the student is to achieve an adequate understanding of biological processes. It is necessary to bear in mind that biology is the area of specialization for dairy production. It may be that specialization for dairymen should be business administration, with a minor in dairy production. The important factor, it is believed, is that we make certain that our students have some area of specialization and not merely a smattering of training.

It is highly important that beginning courses in the sciences be standardized courses. Many freshmen have no well-defined notion of what their eventual career will be and, in fact, have not been in the position whereby they could make such a decision intelligently. Though we hope that those who indicate a preference for dairy production will stay in this field, it can only lead to resentment if severe penalties in the form of additional course work are associated with a change of major. A fair number of our students eventually transfer into human or veterinary medicine. That this can be done with a minimum of penalty is a good inducement for students choosing the dairy production curriculum at the time of enrollment. Thus, we probably gain rather than lose students by providing them with standardized courses which are acceptable in a wide variety of biological fields.

It is believed that our responsibility is to spend considerable time with freshmen, to make it clear to them why we have the requirements in basic sciences. If they understand this fact thoroughly, they will take basic courses more enthusiastically. It is true that, for the most part, returning veterans with greater maturity do more fully understand the necessity for a strong basic science background. Thus, it becomes our duty to advise students with less maturity on this point.

Some departments of our institution have two curricula—one for those planning to enter production fields and one for those preparing for graduate study. We have never had a student in dairy or livestock production who, as a freshman, knew that he wanted to take graduate work. Consequently, it seems advisable to have only one curiculum, and to set this up specifically for those intending to enter the production field. The suggested curriculum was formulated on this basis.

Because of the relatively small number of students enrolling in agriculture, there has been a tendency at California to set up curricula in agriculture in which the basic science requirements have been seriously reduced. Though the number of students is a serious problem, from the standpoint of college administration, it is difficult to believe that this is the answer.

To provide sufficient time for the student to be well-grounded in the sciences, and to have some time for cultural education, it will be necessary to scrutinize carefully, and require as sparingly as possible, the number of courses in the major. It must be remembered that a considerable amount of information which was formerly given in courses in dairy production is now given in courses in animal physiology, animal breeding, physiology of reproduction, animal nutrition, milk secretion, etc. Though duplication is not objectionable, the time which a student has available must certainly be given serious consideration, when considering requirements for his major.

You will note the addition of electives in cultural courses, under Miscellaneous Requirements. Often the curricula in agriculture have been loaded with professional courses, to the extent that the student has had no opportunity to study in areas having no direct relationship to his profession. Much has been written on this topic concerning other professional curricula, such as enginering, and it is interesting that industry itself has been the most vocal proponent of a more libera! education.

Culture is defined by Webster as, "Enlightenment and discipline acquired by mental and moral training." As there is a host of courses which could provide the incentive for meeting this objective, it would seem wiser to allow the student to decide in which areas he wishes to be enlightened.

The number of electives available under the proposed curriculum is too small. Better students, however, will be able to take a larger number of units than is indicated. Furthermore, more of our students are electing to take one or two additional semesters of study, either as an undergraduate or as a graduate. Formerly, it was impossible for the average graduate to take postgraduate work, because of the high requirements of the Graduate Division. Now it has been made possible for the "C" student to enroll in the Graduate Division "without reference to an advanced degree", providing he has an acceptable plan of study. Graduates wishing to widen their training can do so, even though their scholastic record is not sufficiently outstanding to proceed toward an advanced degree.

An important part of the training of any student is the practical experience he obtains. In the suggested curriculum, this is not a requirement but merely a recommendation. There will be a diversity of opinion on this point, but one hesitates to make this an absolute requirement, because we have some students who can not be barred from entering the curriculum but whom we would hesitate to recommend for any dairy farm. We do try to emphasize to freshmen, however, that we will refuse to give them a strong recommendation following graduation if they do not have sufficient practical experience. If we were able to choose the students whom we accept in the dairy production curriculum, we would have no hesitaney in making this a requirement, rather than a recommendation. Our institution has spent considerable time and money in setting up provisions for "on-the-farm" training, and we believe the system, in general, is working very well. The biggest problem is to find opportunities for employment where the student can get wide experience. As dairying becomes more specialized, this problem is accentuated. In any case, we feel that this

problem cannot be answered by classroom instruction.

Function of the Department of Dairy Production in Teaching

Closely related to the problem of the dairy production curriculum are the over-all responsibilities of dairy husbandry departments in teaching. In addition to teaching students in the major, the department has a definite responsibility for nonmajors. As mentioned above, the dairy production curriculum may not be the most desirable one for all students interested in dairying as a profession—a major in business administration, with a minor in dairy production, is a possible alternative.

To attract students wishing to take more courses in other areas, some dairy production departments have watered-down their basic science requirements to the point that, at best, the dairy production courses can be only a glorified extension of high school dairy production courses. Rather than take the heart out of the basic science requirements in the dairy production curriculum, it is suggested that students not wishing much science, major in some other field, and minor in dairy production. We are now providing production courses specifically for nonmajors. Thus, it is possible for us to maintain a high level of instruction in our production courses for our majors, and still allow nonmajors to obtain some training in this field.

Summary

An attempt has been made to outline methods whereby students in dairy production can attain necessary specialization in the biological sciences, without ignoring the importance of a liberal education. Our aim should be to train dairymen in a manner which, in addition to giving them competence, will enable them to take their place in society and to provide intelligent leadership.

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THE DAIRY MANUFACTURING CURRICULUM

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The problem of properly educating our youth has been ever with us and no doubt will long remain. A broad perspective is advisable for those responsible for preparing educational programs for young people at the college level. The destiny of those who select the Dairy Manufactures curriculum lies as a heavy responsibility on the shoulders of the faculty. It behooves us, therefore, to think carefully of the course of study we plan for our college students. The training that may have been satisfactory at the beginning of World War II is largely inadequate in 1957. Our political, economic, and scientific world has changed more in the past 15 years than in any other equal period of time in our history. The remarkable advances both in basic and in applied sciences during this period would be beyond the realm of comprehension to a Rip Van Winkle of today arousing from a peaceful 20-year slumber. There will be no place in tomorrow's top-notch professional circles for the man with limited education and training.

Our Association members, for the most part, are concerned with teaching in institutions of higher learning. Each year brings an increase in the number of new facts concerning the various subjects taught in our universities. This knowledge becomes greater with each year's accumulation of complicated facts, and there will be no end to this expansion of the intellectual horizon. The only way teachers can keep up with educational progress is continuously to streamline their courses and constantly raise the level of standards required of students. An increase in the number of colleges teaching material at a secondary level and a transfer of certain courses from the major universities to the secondary colleges may be the eventual outcome of this trend.

If this happens, you do not need to be told where curricula in dairy sciences will be relegated, if by that time we are not completely divorced from the trade-school approach to teaching. Filling our instructional staffs with people with Ph.D. degrees is not enough to prevent the finger of suspicion from being pointed our way. Our training courses must be such that our graduates will be capable of doing research at a high level, or they must be sufficiently well trained to become promising candidates for understudies in important administrative positions in the large automated food factories that will domiante our industry of tomorrow. There will be no place in tomorrow's institution of higher learning for

faculties concerned primarily with teaching facts commonly known to practical plant operators, such as how to increase overrun in ice cream, how to prevent ice cream shrinkage, cream feathering, or the fishy flavor in butter, or how to make 94-score Cheddar cheese and butter. Big companies will not even look to the large universities for young men trained in such skills, even though such persons might serve temporarily as a convenient and cheap source of supervisory labor at the lower levels. The rules of organized labor and the perfection of automation will make such approaches neither possible nor desirable. As the food companies become larger through growth of the industry and through merging, technical know-how will become concentrated in the hands of relatively few people.

Our job in teaching students in the food industry, if we are to continue doing so, will be to train them in such a manner that they will qualify for the top-notch positions, after having served the necessary training period in preparation for the responsibilities of leadership. To do this job properly, we must avoid curricula with watered-down training in the fields of science, engineering, and commerce. This will necessitate a shift of emphasis from the agricultural college to departments where courses in the basic arts, sciences, engineering, and business management are taught. We must share with the faculties of the supporting departments the responsibility of preparing dairy manufacturing students for successful scientific or managerial careers, and we should consult with these departments regarding our problems, since they will do an increasing amount of the teaching of students majoring in Dairy Manufactures.

What Do You See Ahead?

The dairy plants operating in the period just after World War I bear little resemblance to the food factory and its environment as it will be in the year 1975. What was adequate training of students in the early twenties will be entirely inadequate for those entering the food industry 20 years hence. Let us speculate a bit about the type of living we will be exposed to in this coming era. It is necessary to do this, if we are to assume the responsibility of planning the successful training of the oncoming generation of students.

In this connection, Brig. General David Sarnoff (*Reader's Digest*, April, 1957, page 85), Chairman of the Board of the Radio Corporation of America, makes some pertinent remarks. Certainly, he is in excellent position to visualize what tomorrow has in store for us, considering his past and present professional attainments. His predictions of things to come seem well-founded scientifically, and his opinions are shared by many students of the physical sciences.

He points out that although we have a reasonably good reserve supply of coal, the supply of oil looks much less certain. Nuclear and solar energy, therefore, will be utilized for a large portion of the power necessary to operate our industries and to heat and light our homes. Travel commonly will be done by air, at a much more rapid rate and with greater safety than today. Heavy freight, including perishable foods such as fruits, vegetables, milk, and dairy products, will be quickly and cheaply transported to consumers located thousands of miles from scenes of production. Businessmen with headquarters in Chicago will be able to leave their offices in the morning via private jet planes, for a conference with company-owned subsidiaries located in Bolivia or Honolulu, and return the same day.

It will be a simple matter for a plant superintendent to sit in his office and talk directly with his milk suppliers or his tank milk haulers en route to the factory, and to check an operator or operation in a remote part of his plant or in plants in other cities by means of radio-television. Salesmen, using the same sort of device, will be able to discuss their companies' products directly with prospects. Factories will be illuminated day and night with cold light, which will add comfort to the workers and will reduce fatigue. Heat pumps will be used to supply regulated temperatures in our homes and factories the year around.

We will live in an electronic age. Words will be translated into any desired language by the push of a button. Chemical analysis will be made in the same manner. Both complicated and simple mathematical calculations will be done electronically. Such devices are even now well beyond the experimental stage. Offices will be operated and controlled by automatic machines.

Scientists will continue to become more specialized both in training and in its application. The term dairy chemist in its present meaning will cease to exist. We will have students trained as specialists in the physical and chemical sciences who will deal with only one aspect of the basic elements of milk. If the cost of milk production on the farm cannot be sufficiently lowered, a similar beverage will be synthesized through the combined efforts of research groups not unlike those that produced the atomic bomb.

Waste motions of plant operations will be eliminated through the complete mechanization of all processes. Push-button control will, of course, be the order of the day. Milk and its products will be automatically processed, packaged, and stored. Factories will be removed from the city to rural districts located closer to supplies and labor. Extended shelf life of all products will make possible infrequent deliveries to the large supermarkets which, for

the most part, will control the retailing of milk and its products. Warehousing probably will become a function of the retailer. Deliveries of food products to such warehouses by air will be practiced by factories located hundreds of miles distant, at points of low production costs. The availability of raw materials at the right price and quality may become more of a factor in determining the location of the factory, because of the relative cheapness of transportation resulting from application of atomic energy.

Labor will be highly paid but will also be highly productive. The training of technicians will become a function either of the large corporation or of the secondary school set up for this purpose by the state or federal government. In training these workers, emphasis will be placed on practical engineering and the social sciences.

Laborers will be limited to 30 hours of work weekly. To properly adjust them to their work environment, to the profitable use of their leisure time, and to the retirement from active duty which will come to age 60 or before, each corporation will need to employ specialists in applied psychology.

Both basic and applied research will be carried on by the large corporations, but by separate groups in separate locations. Products will become so standardized in both composition and quality that growth from new business for the large corporations will stem largely from their ability to outdo their competitors in advertising, salesmanship, creation of new products, and diversification. Increased profits will depend not so much on business expansion as on the ability to control operating costs, through greater application of automation or the development of improved machines and methods.

Public relations will grow in importance each year, and the food corporation of 1975 will need to employ people capable of establishing a good name for the company and its products. In the coming battle for public approval for brand names, expert showmanship will be required. Public relations experts will be in demand to sell their profit-minded corporations to the critical public, whose support for big business is vital if free enterprise is to survive. The person heading the public relations department of tomorrow, because of his importance, will have "cabinet rank" and probably will supervise not only the public relations chores but also advertising and selling, since all three functions are interrelated.

Tomorrow's dairy industry will be closely watched and controlled by the federal and state governments. Already there are many laws regulating labor, markets, plant construction and operation, sewage disposal, labeling, advertising, trade practices, composition and purity of products, and chemical additives. As society becomes more complicated, populations become more congested, and competition becomes keener, we can expect even more regulation of industry and society in general. It becomes a matter of "the greatest good for the greatest number." Therefore, as we move into this new era of tomorrow, legal problems gain significance, and a legal staff, trained also in the techniques of food manufacture, becomes almost as vital a part of the large dairy corporation as is the laboratory control.

Tomorrow's Curriculum

The big question is, how should we prepare students with a major in Dairy Manufactures (technology or industry) for the industry of tomorrow? Is our present approach the correct one? How well will our graduates, trained as they are today, fit into the scheme of things to come? How long will they be willing to stay with the large corporations, if they find their training does not carry them far up the ladder of success? Can a young man as now prepared for life, in curricula loaded with trade-school type courses, expect to go much farther than the man who pushes the button in tomorrow's dairy plant? How about the student who aspires to do more than push that button? Can he get the training he needs in electronics, mechanical and electrical engineering, heat transfer, refrigeration, law, psychology, personnel administration, sales, advertising, physics, statistics, biochemistry, higher mathematics, foreign language, economics, social sciences, and advanced chemistry, if he is required to take courses in soils, livestock judging and management, tree pruning, fluid milk, butter, cheese, ice cream, condensed milk, and milk powder manufacture? Should we not think in terms of educating our students rather than of training them?

We need to redefine our objective in Dairy Manufactures. If we continue to train young men primarily for plant supervisory work, we must reconcile ourselves to two things: *(1)* We will find it increasingly difficult to recruit high-caliber students who will stay with the dairy industry upon graduation, and *(2)* eventually the teaching of Dairy Manufactures will be relegated to the secondary schools. On the other hand, if we hope to attract high-caliber students with the idea of training them for top-level positions in the dairy industry, we will have to change our curricula so as to make it possible for students to become well trained in: *(a)* food engineering, *(b)* basic food sciences, or *(c)* business management. To make it possible for a student to secure the necessary background in the dairy industry, cultural courses, and any one of the three basic fields of application (food engineering, food sciences, or business management), we need to eliminate all general agriculture courses from our curricula and replace the five

or more product courses we now require with only one or two courses dealing primarily with the basic functions common to all processing of products. We will need to continue to give the student expert training in chemical and bacteriological laboratory control and product evaluation. In this way, a young man can be both trained and educated and will be better prepared to meet the problems of tomorrow.

A college student's time is both limited and costly, and we, the faculty, are obligated to make the best possible use of that time. How can we justify using hours of the student's schedule teaching him how to churn butter; make cheese; pasteurize and bottle milk; wash bottles, cans, and vats; scrub floors; make mix; freeze ice cream, and operate the vacuum pan and drier? These are things he can learn during his summer vacation or during the training period given him by his first employer. A chemical engineer with a basic training in heat transfer, refrigeration, and physics can pick up the technique of such operations in short order. Why, then, should we feel obliged to teach such practices to college students? The conclusion is obvious. If we train and educate our students properly, there will be little time for laboratory practices in the manufacture of dairy products. Industry itself is in best position to supply the practice training needed and can do it at less cost to the student.

How, then, should students interested in going into the dairy field be trained? Because of the immaturity of most college freshmen, the first year should primarily be one of orientation, in which the student passes off certain general university requirements, gets his first courses in English, mathematics, and chemistry, and a survey course in the dairy and food fields. By the end of this year, each student should be given aptitude tests, and upon the recommendation of a competent adviser should be encouraged to select from one of the following three submajors:

1. Research and laboratory control
2. Business management
3. Food engineering.

The first group will appeal to students interested in chemistry, higher mathematics, and microbiology. Many in this group will pursue graduate training and will prepare themselves for research at a high level and possibly for collegiate teaching. Others in this group may go into plant laboratory control work.

For the second group—those interested in management—major emphasis will be placed on College of Commerce courses in business law, business letter writing, principles of economics, corporation finance and management, personnel administration, sales, advertising, business administration, and labor management. This group will attract most of the students who register in Dairy Manufactures. The program will enable them to take essentially the same work as a commerce major.

For the third group—the food engineers—the emphasis will be placed primarily on courses offered either by the Department of Agricultural Engineering, the College of Engineering, or the Department of Chemical Engineering. Emphasis will be placed on mechanical, electrical, and architectural engineering, and possibly physics. This group will need to be given rather wide latitude, for their fields of application will vary. Some will want to concentrate on some form of applied automation in food plants. Some will want to do research in developing machines and methods for furthering automation. Some will want to become sales engineers.

Our course requirements for graduation should be rather loosely held, for it is conceivable that it would be better for some students to browse in two, or possibly all three, of the fields of specialization indicated.

In addition, there should be certain requirements in humanities for all majors in Dairy Manufactures. This would include courses in sociology, psychology, logic, and political science.

The question might be justly raised as to whether or not such a program can be completed in four years. Possibly five years will be required. However, this should not be considered a drawback. Our job is to train and educate students properly. It takes more than four years to train a doctor or a lawyer. It takes seven years to train a student for teaching and research at the college level. It is the objective that should determine the years of college education necessary.

Possibly we in Dairy Manufactures would be justified in raising our sights a bit, if by so doing we could attract better students and do a better job of preparing them for life. If it takes five or even six years to accomplish this objective, well and good. Our goal at the college level of teaching should be to educate students for industry leadership—not to train them to be workers in the "vineyard."

OUR ASSOCIATION

REVIEW OF THE UNITED STATES' RELATIONS AND ACTIVITIES WITH THE INTERNATIONAL DAIRY CONGRESS

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The purpose of this paper is to review for the membership of the American Dairy Science Association the organization and activities of the International Dairy Federation and the International Dairy Congress. This review is appropriate, in order that the membership will understand more fully the reasons why the Association has, on different occasions, given consideration to becoming affiliated with the International Dairy Federation and has had committees appointed to study this possibility. This article and the report on page 1381 are the results of the work of a current A.D.S.A. committee which has been investigating this subject.

The International Dairy Federation—IDF

The International Dairy Federation is an organization that has for its objective the promotion through international cooperation of the solution of scientific, technical, and economic problems in the international dairy field. In carrying on its program, it does not interfere with domestic matters of any country. It was organized in Brussels, Belgium, in 1903, through the initiative of the Belgian National Dairy Society, at the time that Society arranged the first meeting of the International Dairy Congress. The home office of the International Dairy Federation has always been in Brussels.

The IDF presently is made up of 22 member countries, including Australia, Austria, Belgium, Canada, Denmark, Finland, France, West Germany, India, Ireland, Israel, Italy, Japan, Luxemburg, Netherlands, New Zealand, Norway, Russia, Spain, Sweden, Switzerland, and the United Kingdom. The recommended membership for each country is a national committee representing the different dairy groups of that country, such as production, manufacture, commerce, science, and administration. However, the membership of several of the countries is maintained by the government. Each national committee is entitled to five members who are privileged to participate in the General Assembly. However, each country is entitled to one vote only in Federation affairs.

The General Assembly of IDF

The governing body of IDF is the General Assembly, which is composed of up to five members from each member country, and the President, three Vice-Presidents, and the President of the Commission of Studies, who is ex officio. The Assembly elects the President and three Vice-Presidents, and the President of the Commission of Studies. The Assembly appoints the General Secretary, establishes the Executive Committee, and appoints members thereto, and acts on all proposals relating to the activities of the IDF and on all proposals of the Commission of Studies. The Assembly holds meetings at least once a year.

The Executive Committee of IDF

The Executive Committee is composed of the President, the three Vice-Presidents, the President of the Commission of Studies, and seven members from the membership of the General Assembly, one of whom must be from Belgium. The membership must represent at least eight different countries. The Executive Committee submits proposals to the General Assembly for its deliberation, is responsible for acting on decisions adopted by the General Assembly and for the administrative and financial management of IDF.

The General Secretary of IDF

The General Secretary, a Belgian, is responsible for the organization and direction of the secretariat, is concerned with the finan-

[1, 2] Chairman and members of A.D.S.A. Committee for investigating affiliation of A.S.D.A. with the International Dairy Federation.
[3] Chairman, U. S. Delegation to the XIVth International Dairy Congress, Rome, Italy, 1956.

cial, administrative, and office work, and serves in the usual capacity as Secretary to the Executive Committee and the General Assembly for the IDF business affairs.

The Commission of Studies of IDF

The Commission of Studies is a permanent body established by the General Assembly, and is charged with the general scientific, technical, and economic examination of all dairy problems. The Commission in turn reports to the General Assembly.

Much of the work of the IDF is done by and through this Commission of Studies. The Commission may establish special commissions to make detailed investigations of special dairy problems, and appoints the president and members of the special commissions which usually are made up of members representing national committees of the various countries. With the approval of the General Assembly, the Commission of Studies may organize competitions, special scientific meetings, and arrange for publication of books, documents, proceedings of meetings, and conferences relating to activities of the special commissions.

At the present time, there are nine special commissions studying various problems. These commissions are: International Commission on Dairy Economics; International Commission for Standardization of the Methods of Analysis for Milk and Milk Products—Chemistry; International Commission for Standardization of the Methods of Analysis of Milk and Milk Products—Bacteriology; International Commission for Cheese; International Commission for Dried and Condensed Milk and Milk Products; International Commission for Dairying in Warm Countries; International Commission for Hygienic Production and Control of Milk; International Commission on Industrial Dairy Techniques, and Special Commission of the Countries Signatory to the Stresa Convention. In addition, the Commission of Studies proposes and organizes special work conferences and seminars on various problems. Summaries of the accomplishments of the activities of these special commissions and meetings are periodically issued from the secretariat of IDF. The IDF, usually through the Commission of Studies, collaborates on projects or meetings with other international organizations, particularly the Food and Agriculture Organization of the United Nations, with which it enjoys special consultative status.

The International Dairy Congress

Another primary activity of IDF is to sponsor International Dairy Congresses. Congresses are held about once every three years in one of the member countries. The first Congress was held in Brussels, Belgium, in 1903. The 14th and most recent Congress was held in Rome, Italy, in 1956. While Congresses are held under the auspices and approval of IDF, it is the job of the National Committee of the country in which the Congress is held to organize and put on a Congress. The National Committee undertakes this task with the sanction of the local government and the cooperation and assistance of the local dairy industry groups.

A Congress meeting usually consists of a four- or five-day meeting during which various sessions are held for the discussion of technical, scientific, economic, and trade topics relating to the world's dairy industry. In recent Congresses, the sessions have been classified into three broad sections: one dealing with milk, its production, handling and marketing, nutritional value, and sanitary quality; the second, with technical and economic problems relating to dairy products; and the third, with milk and dairy products, methods of analysis, and control measures, dairy legislation and trade. Sepcial conference lectures and symposium are also held and various exhibits, tours, and related functions are sponsored before, during, and after the meetings.

Dairy specialists of member countries, and nonmember countries as well, are invited to submit papers to the Congress covering subjects included in the agenda. The papers submitted are collected and are included in the Congress proceedings, which are published and made available at the time of the meeting. Papers included in a particular subject are not presented as such at the Congress, but a specialist is appointed to review the collection of papers dealing with a common subject matter. The reviewer, or rapporteur, presents a paper covering the information contained in the group of papers he has reviewed. He also includes his own information and ideas in this review. Following this report, the subject is discussed by a few selected discussion leaders and then the discussion is opened to the participants in attendance at the sessions. These reviews and discussions are also included in the proceedings. The abstracts of papers, the discussions and related information, are made available in the proceedings in several languages, usually English, French, German, and Spanish.

Participation of the United States in International Dairy Congresses

The Congress meetings are open, and dairy specialists from all countries are privileged to register and attend. The countries that are members of IDF are also represented by official delegations. In addition, the government of the host country, with the concur-

rence of IDF, extends official invitations to the governments of non-IDF member countries to have official delegations in attendance. Quite a number of countries send delegations on this basis.

Although the United States has never been a member of IDF, it has cooperated with the host country by sending an official delegation to each of the International Dairy Congresses. Members of the delegation are recommended by the Secretary of Agriculture and appointed by the Department of State.

It seems appropriate to describe the manner in which the United States has participated in these Congresses. Well before the time the Congress was to be held, the government of the host country, through its Ministry of Foreign Affairs, has sent an official invitation through its Embassy to the U.S. Department of State. The Department of State has referred the invitation to the U.S. Department of Agriculture for a recommendation. The Department of Agriculture has repeatedly considered the technical aspects of this Dairy Congress worth while, and has recommended to the Department of State that the invitation be accepted and that an official U.S. delegation, representing the technical phases of the dairy industry, be in attendance. The Department of State, on concurrence, has accepted the invitation and indicated to the host government that a U.S. delegation would be represented at the Congress meeting.

United States Liaison Officer

Thereafter, the Secretary-General of the Congress Organizing Committee has requested of the United States that an individual be designated as liaison to develop U.S. participation at the Congress. The liaison designated has usually been a dairy science specialist, or a committee of specialists, located in the U.S. Department of Agriculture. This liaison has assumed the responsibility of giving publicity in this country to the forthcoming International Dairy Congress meeting, of inviting technical workers to submit papers for inclusion in the Congress proceedings, and to perform other duties relating to activities in connection with the Congress meetings.

The Official U. S. Delegation

When the time comes for the Department of State to organize the official delegation, it has looked to the Department of Agriculture for proposals. The Secretary of Agriculture has recommended to the Department of State the membership of the official delegation. In formulating these recommendations, the Department of Agriculture has given consideration to proposing a delegation that would represent recognized technical workers in dairy science and teaching, in the Department of Agriculture and in the land grant colleges and universities, members of the American Dairy Science Association, and representatives from various phases of the dairy industry. Attempts have been made to have various sections of the country represented on delegations. The liaison officer usually has been designated the chairman, and the Agricultural Attaché of the U. S. Embassy in the host country secretary, for the official U. S. delegation. The Department of State has invariably accepted the recommendations of the Secretary of Agriculture for the delegation membership. The Department of State always has financed from the International Conference Fund the expenses of the delegation chairman and a small number of the delegates. The other members' expenses either have been paid by the organization that employs them or have been handled personally.

The United States always has sent strong delegations to the Congresses. While at the Congress, the delegates participate freely in the sessional meeting and give freely and effectively of their knowledge and experience. Since the delegation members are guests, they do not participate in any official decisions or recommendations. Following each Congress, the delegation makes detailed formal report to the Department of State covering the Congress deliberations and actions and the activities of the U. S. delegation while at the Congress.

It is fair to say that delegates who have been to recent Congresses feel that it is important that the United States be in attendance in an official capacity. There is some concern that the Congress program is too general and is drifting away from the technical and scientific aspects of dairy production and manufacture problems. Others lament the fact that there is too little included in the agenda on the production side of dairying. However, the contacts made, the information received, and the information given make the participation highly valuable to this country.

American Dairy Science Association—International Dairy Congress Relationship

Members of the American Dairy Science Association have wondered frequently why the Association has not participated more actively in IDF affairs and the International Dairy Congresses. To do so the Association would need to become a member of the IDF. The IDF on several occasions in the past has solicited the A.D.S.A. for a membership and the A.D.S.A., through special committees, has investigated the matter in detail on two recent occasions, in 1948 and 1952. At the

request of IDF, the Association has reviewed the situation again this year and the Executive Board has approved the committee recommendation that A.D.S.A. not affiliate with the IDF. The compelling reason why A.D.S.A. cannot affiliate with IDF is basically because the interest and activities of the latter are beyond the scope of interest of the A.D.S.A.

In addition to the A.D.S.A. special committee's review, a number of dairy industry trade associations in the United States have been engaged jointly in determining the feasibility and desirability of becoming affiliated with IDF. A group of dairy leaders representing different organized groups has investigated the possibility of forming a National Committee to become a member of IDF. However, at a recent meeting in Washington, D. C., of representatives of several dairy associations, a decision was reached that, owing apparently to lack of interest and financial means, the formation of a National Committee to take out a membership in IDF be deferred to the indefinite future. A report of the deliberations of these representatives appears on page 1381.

Report of the Special Commitee to Investigate the Desirability of the American Dairy Science Association Affiliating with the International Dairy Federation

President C. F. Huffman appointed a special committee to study and report to the Executive Committee on the desirability of having the American Dairy Science Association affiliate with the International Dairy Federation. This committee consisted of R. E. Hodgson, Chairman, I. A. Gould, and G. M. Trout. All were official United States delegates to the XIVth International Dairy Congress held in Rome, Italy, September 24 to 28, 1956.

In addition to the meetings of the Congress, this committee had the opportunity to meet with the officers of the International Dairy Federation, at the Federation's request, at which time the latter outlined and discussed the structure, objectives, and working procedures of that organization. The Committee has had the further advantage of studying the Federation Statutes, as well as reports of the activities of the nine Special Commissions of the Commission of Studies.

As a result of their studies, the Committee reached the following conclusions:

(1) While the International Dairy Federation concerns itself with scientific, technical, and educational phases of dairying, it also has interests and carries on activities beyond the scope and interest of the American Dairy Science Association.

(2) Reasonably full participation in the activities of the International Dairy Federation involving *(a)* attendance of members at the Congresses, at annual meetings of the Federation, and at meetings of the several Study Commissions, and *(b)* membership fees, would require funds far beyond the resources of the American Dairy Science Association. It is estimated that to join and participate actively in the affairs of the IDF would require an annual budget of from $16,000 to $20,000.

(3) For these reasons, the Committee recommended that the American Dairy Science Association not consider taking out a membership in the International Dairy Federation by itself.

The International Dairy Federation recommends that membership in the Federation be maintained by a National Committee representing different phases of the dairy industry within a country. There has been some interest on the part of several of the dairy industry trade associations in the United States in becoming affiliated with the International Dairy Federation. A study of this possibility was made recently by several of the major dairy associations. A representative of A.D.S.A. participated in these studies. The American Dairy Science Association, because of its interest in the scientific, technical, and educational phases of the Federation, and by reason of the opportunity for service in this area, might well consider the possibility of joining with other organizations in forming a National Committee that would maintain a membership in the International Dairy Federation. The International Dairy Congresses certainly are of interest to A.D.S.A. and in the past many of its members have contributed, by attendance and participation as individuals, or as official U.S. delegates, in the Congress meetings. A.D.S.A. members also could render service to the Federation's Special Study Commissions.

Because of these interests the Committee recommended:

(1) That if there was sufficient interest among the several dairy organizations in the United States to form a National Committee and take out a membership in the International Dairy Federation, the A.D.S.A. would be willing to join with these groups as a member of this Committee.

(2) That it be understood by all those who may become members of the National Committee that A.D.S.A. would not be in a position to contribute funds in support of the cost of membership and participation, and that this cost would necessarily be borne by the other member organizations.

(3) That as a member of the National Committee, A.D.S.A.'s main contributions would be in the scientific, technical, and edu-

cational phases, and it would be hoped that the National Committee would depend heavily on A.D.S.A. to represent this country in these aspects of the IDF membership responsibilities.

In conclusion, the A.D.S.A. Committee recommended that the A.D.S.A. should take formal action to express appreciation to the officials of the International Conference Division, Department of State, for the excellent support that that office has given in providing funds to send official delegates to past Congresses, and to urge on them the desirability of continuing this support as a primary means whereby scientists from the state colleges and universities and the Department of Agriculture may attend and participate in future Congresses.

REPORT OF MEETING OF REPRESENTATIVES OF AMERICAN DAIRY INDUSTRY ASSOCIATIONS, RELATING TO THE UNITED STATES AFFILIATING WITH THE INTERNATIONAL DAIRY FEDERATION

R. E. Hodgson

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During the meetings of the 14th International Dairy Congress held in Rome, Italy, September 24-28, 1956, the officers of the International Dairy Federation (IDF) invited the members of the Official United States Delegation to a meeting, for the purpose of outlining the objectives and program of that organization and to solicit the affiliation of the United States with the Federation. The recommended type of country membership is a national committee composed of representation from all segments of the industry, including production, manufacture, science, commerce, and administration. The present membership comprises 22 countries.

The officers of IDF were advised that the United States delegation would study the proposal and, if possible, ascertain the interest of the major associations of the American dairy industry in forming a national committee and taking out a membership.

At a meeting of the United States Delegation in Rome, the members instructed its Chairman, R. E. Hodgson, to assume the responsibility for the group, in taking the necessary steps to acquaint the major dairy industry associations with the objectives and program of IDF, and to solicit their interest in the membership proposal; and further, to provide the opportunity for these associations to come together and formulate such plans and actions as they considered desirable in relation to the invitation to membership in IDF. In carrying out these instructions, R. E. Hodgson secured from IDF copies of the Federation statutes and other material relating to reports, etc., issued by IDF, and made them available to dairy associations. He also provided them with an abstract of the organizational structure of IDF, a summary of its activities, interests, and programs, together with a list of items that associations might wish to consider in arriving at a decision as to the extent of their interest in IDF. This material was sent to the following 15 dairy organizations and to the members of the U. S. Delegation: American Butter Institute, American Dairy Association, American Dry Milk Institute, American Dairy Science Association, Dairy Industry Committee, Dairy Industry Supply Association, Evaporated Milk Association, International Association of Ice Cream Manufacturers, International Association of Milk and Food Sanitarians, Milk Industry Foundation, National Association of Artificial Breeders, National Cheese Institute, National Dairy Council, National Milk Producers Federation, and National Purebred Dairy Cattle Association.

Following this, a meeting was called in Washington, D. C., on May 23, 1957, to which representatives of these associations, the members of the U. S. Delegation, and other interested individuals were invited, the purpose being to discuss jointly the question of United States affiliation with IDF and to formulate plans to carry out decisions arrived at. The following individuals attended this meeting: C. M. Fistere—American Dry Milk Institute; M. H. Brightman—Dairy Industry Committee; R. Everett—Dairy Industry Supply Association; G. W. Weigold—Dairy Society International; D. H. Williams—International Association of Ice Cream Manufacturers; E. B. Kellogg—Milk Industry Foundation; E. M. Norton—National Milk Producers Federation; M. Gebert—National Milk Producers Federation; J. H. Remsberg—Purebred Dairy Cattle Association; C. J. Babcock—Foreign Agricultural Service, USDA (also representing International Association of Milk and Food Sanitarians); D. R. Strobel—Foreign Agricultural Service, USDA; H. L. Forest—Agri-

cultural Marketing Service, USDA; H. C. Trelogan — Agricultural Marketing Service, USDA, and R. E. Hodgson—Agricultural Research Service, USDA (also representing American Dairy Science Association).

Each association representative presented his views of the question and the interests of his association. A full discussion took place relating to the functions of IDF, the problems involved in U. S. membership, costs, advantages, and disadvantages to the United States affiliation with IDF, etc. As a result of these discussions, it was apparent that there was insufficient interest among the various associations to join together to form a national committee and to support the cost of affiliation with IDF. It, therefore, was recommended that the idea of the United States dairy industry joining IDF be dropped for the present.

The discussion further revealed the fact that many individuals, associations, and companies in the dairy industry in the United States are members and support the objectives and program of the Dairy Society International. In recognition of this fact, the group went on record to resolve that steps be taken within the United States dairy industry to support the Dairy Society International in its worldwide promotion to enlarge utilization of milk in all its forms, and in its efforts to advance dairy science and dairy education. The group further went on record as recognizing the merits and important functions and activities of the International Dairy Federation, and urged that appropriate steps be taken to encourage a developing of mutually beneficial cooperation between the International Dairy Federation and the Dairy Society International, to facilitate cooperation in a more rapid worldwide dairy progress.

The group suggested this report be sent to the associations and individuals invited or in attendance at the May meeting, to the members of the U. S. Delegation, to the International Dairy Federation, to the Dairy Society International, and to the Food and Agricultural Organization.

OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Status of the Problem of Diet and Coronary Heart Disease

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The primary cause of death among adults in the United States is arteriosclerotic and coronary heart disease. At a given age, more men and more women die from heart disease in the United States than in other countries for which statistics are available. Some clinical and actuarial evidence indicates that the incidence of the disease is increasing. The most frequent deaths at earlier middle age occur among our white adult male population.

This problem has captured public interest, because it touches so closely upon the lives of everyone. The medical profession is intensely interested, because of the magnitude of the problem as it confronts them in their daily practice. The dairy industry and all food industries are vitally concerned because diet, particularly dietary fat, has been suggested as one possible contributing factor in the development of atherosclerosis, the underlying disease responsible for coronary heart disease.

The Nature of Coronary Heart Disease

In coronary heart disease, insufficient flow of blood through coronary arteries deprives some segment of heart muscle of oxygen and nourishment. The underlying cause is atherosclerosis. This is a slowly developing disease of the arteries, in which the inner lining of the wall of the blood vessel thickens from one to many layers of cells. Cholesterol, phospholipids, fats, and proteins are deposited beneath this thickened lining with formation of a mass (atheroma) which protrudes into the lumen of the vessel. In late stages, calcium salts are deposited in the atheroma, "hardening the arteries," in a disease called arteriosclerosis.

As atherosclerosis progresses and the atheroma grows, it reduces the opening through which blood flows. Hemorrhage may occur in the atheroma and cause additional swelling. A blood clot may form at the surface of the injury, further closing the blood vessel. Such a clot is called a thrombus. Its occurrence is called thrombosis. The event is called coronary thrombosis when it happens in a coronary artery. The resultant block to blood flow beyond the site is referred to as coronary occlusion. The person is said to have had a heart attack.

Most heart attacks are not fatal. If supply of blood is only partially shut off, the person feels chest pain and weakness until flow of blood is reestablished. Usually, blood flow immediately increases through undamaged coronary arteries which lie near the damaged vessel. Thus, the muscle receives minimum oxygen until a network of blood vessels becomes reestablished. The injury to the muscle cells caused by temporary lack of oxygen is called myocardial infarction. Scar tissue forms as healing takes place. An electrocardiogram will reveal the location and extent of the injury.

If blood supply is completely cut off, cells of the heart muscle die, heart beat is interrupted or ceases, and all cells of the body die from lack of oxygen and nourishment.

The Cause of Atherosclerosis Is Unknown

The precise cause of atherosclerosis is unknown. Also unknown is the set of circumstances which cause a blood clot to form in a coronary artery at a given time.

Many factors may contribute to development of atherosclerosis and subsequent formation of a thrombus. These include: *(1)* Pressures against the wall of the artery from force of blood flowing past; *(2)* structure and function of cells in the artery wall which make them susceptible to changes which occur in atherosclerosis; *(3)* chemical reactions in the artery wall (rate at which certain compounds are synthesized or broken down by enzymes under influence of various hormones); *(4)* materials present in blood which might injure the artery wall (by entering the wall and staying there, or by

causing the cells to form an excess of protein and lipid-containing material which remains in the wall to damage it), or cause a blood clot to form and fail to dissolve.

Some changes in blood components which might affect development of atherosclerosis and ultimate coronary occlusion are: *(1)* Excess of cholesterol, saturated fatty acids, neutral fat, and some lipoproteins; *(2)* deficiency of essential fatty acids or amino acids, protein, vitamin B_6, magnesium, and certain hormones and enzymes; and *(3)* imbalance with respect to nutrients derived from food, and hormones and enzymes produced by the body.

Role of Blood Cholesterol and Other Lipids

One theory explaining development of atherosclerosis suggests that changes occur in level of blood cholesterol, other lipids and lipoproteins, which cause them to be deposited beneath the injured lining of the artery wall; and that this process continues as long as blood levels of these materials remain abnormal. As a result, concentrations of cholesterol and other lipids in blood have been extensively measured in studies of atherosclerosis. This is done in absence of a direct means of determining the presence and severity of this disease in the coronary arteries of living persons.

The validity of using concentration of blood cholesterol and other lipids as a measure of atherosclerosis is based on the following findings: *(1)* Cholesterol is deposited in atheroma; *(2)* individuals who have very high levels of serum cholesterol and/or abnormal patterns with respect to other lipids, have a high incidence of death from coronary heart disease; *(3)* population groups with a high average level of serum cholesterol have a high incidence of death from coronary heart disease; *(4)* cholesterol concentration in the blood increases with age and so does the occurrence and severity of atherosclerosis; *(5)* in certain diseases, such as diabetes mellitus and xanthoma tuberosum, individuals have higher than average levels of blood cholesterol and also are more prone to atherosclerosis and death from coronary heart disease than is the average population; *(6)* feeding cholesterol to experimental animals, with or without hormonal disturbance, usually leads to elevation of blood cholesterol and to the development of atherosclerosis; *(7)* experimental animals with elevated blood cholesterol levels develop atherosclerosis.

On the other hand, concentration of cholesterol in blood and/or pattern of lipoproteins present can not be taken as an accurate measure of existence of atherosclerosis because: *(1)* Many people with low levels of blood cholesterol die of coronary heart disease; *(2)* many people wth high levels of blood cholesterol never develop coronary heart disease; *(3)* coronary heart disease is more common in the United States among white men than among white women, at all ages up to and beyond 65 years, but blood cholesterol levels are not consistently higher in men than in women at all ages when coronary heart disease is more common in men; *(4)* metabolism is disturbed in those diseases and states of hormonal imbalance in which atherosclerosis and high blood cholesterol levels are found. Rather than excess blood cholesterol being the cause and atheroma the effect, both may be the result of the same disturbed metabolism.

How Diet May Be Related

Concentration of cholesterol, lipids, and lipoproteins in blood can be altered by kind and amount of food consumed. Hence, dietary deficiency, imbalance, and disturbed lipid metabolism have been suggested as possibly contributing to development of atherosclerosis and coronary heart disease.

It is reasonable to think of a possible relationship of diet to disease. Tissue damage can occur in a number of diseases through accumulation of an excess of normal body substances. For instance, pyruvic acid accumulates in beriberi in thiamine deficiency. Pyruvic acid accumulates because thiamine is an essential part of an enzyme system which is required to oxidize pyruvic acid to carbon dioxide and water, with release of energy. Increasing carbohydrate in the diet in thiamine deficiency increases severity of beri-beri and increases pyruvic acid accumulation in blood and tissues. Yet, neither carbohydrate nor pyruvic acid causes beri-beri. The cause of this disease is thiamine deficiency.

Calcification of soft tissues with an excess of vitamin D is an example of a disease which results from accumulation of a normal body substance through an excess of a different nutrient. Calcium is normally deposited in bone. With toxic levels of vitamin D it can be deposited in soft tissues, including heart muscle.

The possible influence of deficiency, excess, or imbalance of nutrients in the diet, as contributing to the development of atherosclerosis, is indicated by reports of: *(1)* A high incidence of the disease in countries where food supply is abundant, and a relatively low incidence where food supply is marginal; *(2)* elevation of blood cholesterol levels during weight gain and decrease of blood cholesterol levels during weight loss; *(3)* insurance statistics associating death from heart disease with incidence of overweight; *(4)* more heart disease in some countries and among some groups with a high fat intake than in countries and groups with a low fat intake; *(5)* changes in blood cholesterol levels with intake of different kinds and amounts of fat, carbohydrate, and protein; *(6)* tendency for highly unsaturated fatty acids to lower blood cholesterol levels and for saturated fatty acids to raise them; *(7)* development of atherosclerosis in some animals fed cholesterol; *(8)* protective effect of high levels of good-quality dietary protein against develop-

ment of atherosclerosis; *(9)* increase in dietary requirement of rats for essential (highly unsaturated) fatty acids and magnesium when cholesterol is fed; *(10)* elevation of blood cholesterol in rats deficient in dietary magnesium; *(11)* damage to walls of arteries and heart when calves and rabbits are fed magnesium-deficient diets; and *(12)* recognition of the specific function of various vitamins and minerals in the metabolism of fats, carbohydrates, and proteins—all of which contribute to the synthesis of cholesterol and other lipids and lipoproteins in the body.

Diabetes mellitus is an excellent example of a disease in which metabolism is disturbed and the course of the disease is influenced by diet. The basic problem in diabetes is absence of sufficient amount of the hormone, insulin. Blood levels of glucose and of acetone bodies become easily elevated in diabetics. The higher the carbohydrate intake, the greater the elevation of blood glucose. The greater the intake of fat, the greater the amount of acetone bodies in the blood. Yet, neither the dietary carbohydrate nor fat, nor blood glucose nor acetone bodies, are responsible for this disease. Diabetes mellitus is caused by lack of sufficient insulin.

The concept of atherosclerosis as a disease of disturbed metabolism is strengthened by the fact that it: *(1)* Takes years to develop; *(2)* is seen more often in men than in women, and in white than in other races; *(3)* is greater among diabetics than among nondiabetics; *(4)* develops in some animals only when hormonal imbalance is created; *(5)* can be prevented in some male animals when the female sex hormone is given.

Milk Fat and the Problem

With all of these involved relationships, why is the dairy industry specifically concerned about this entire problem of diet and heart disease?

Milk fat contains cholesterol, as does the fat of eggs and meats. These foods first became involved in discussions of atherosclerosis because of their cholesterol content. The theory was, "If dietary cholesterol elevates blood cholesterol, which in turn causes this material to be deposited in the walls of arteries, then dietary cholesterol should be eliminated." However, as research progressed, dietary cholesterol has been judged relatively unimportant in human atherosclerosis. Varying cholesterol intake from none to very large amounts has little effect on blood cholesterol levels of human subjects.

Milk fat, and all other food fats, became involved with this problem when it was shown that varying total fat intake caused changes in the concentration of cholesterol in the blood of human subjects.

This appeared of special significance when linked with what may be an increase in coronary heart disease in the United States, and with an increase in consumption of fat. However, such increases have been definitely established. Methods of reporting this form of heart disease have changed through the years and, therefore, do not accurately reflect what has happened. Fat consumption is estimated from figures for the amount of fat in foods sold through retail outlets, and such figures do not reflect the amount of fat wasted in food preparation and at table.

Even if these increases and correlations are real, they do not establish a cause-and-effect relationship between dietary fat and heart disease. Neither do they show the relationship of milk fat to our total fat and calorie intake. Actually, *(1)* milk fat from all dairy foods, including butter, contributes only one-tenth of the calories and one-fourth of the fat in our food supply; *(2)* separated fats and oils, excluding butter, contribute more than 40% of the fat in our food supply; and *(3)* our per capita consumption of milk fat has remained almost constant since 1920, decreasing a little in the last few years; whereas, per capita consumption of other fats has increased.

Different types of fats, when fed at high levels to human subjects, have a varying influence on blood cholesterol levels. Generally, the influence of specific fats appears related to the ratio of saturated to highly unsaturated fatty acids present in the fat. Milk fat, fed at high levels, tends to elevate blood cholesterol more than do fats and oils which contain more of the highly unsaturated fatty acids. No one has demonstrated any particular influence of milk fat, when it is fed at levels ordinarily consumed along with other fats in human diets typical in the United States.

When one considers that the United States heads the list in number of deaths from coronary heart disease, but stands 15th in its per capita consumption of milk fat—with a decreasing rather than increasing per capita consumption in recent years—then it becomes highly questionable that the influence of large amounts of milk fat in elevating blood cholesterol has any pertinent relationship to our problem of coronary heart disease. Indeed, differences among countries in rate of deaths from this disease are not quantitatively related to differences among the same countries in per capita consumption of milk fat from all dairy foods.

Because a high level of dietary protein lowers blood cholesterol levels and reduces atherosclerosis in experimental animals, and because human populations which vary as to fat intake do not vary much with respect to per cent of calories from protein, it has been suggested that the amount of fat in the diet is not so important as is the ratio of fat to protein. If this is true, milk fat is in a particularly fortunate position as a dietary fat because: *(1)* For each calorie of fat from all foods consumed in the United States, 0.29-calorie from protein is also consumed; *(2)* for each

calorie of fat consumed from all dairy foods in the United States, 0.52-calorie from protein is also consumed; and *(3)* for each calorie of fat consumed as whole milk, 0.44-calorie from protein is also consumed.

Thus, milk fat carries with it a better supply of protein than is available in our total food supply.

Dietary Recommendations

Atherosclerosis and coronary heart disease are clinical problems. Treatment must be in the hands of the medical profession. Diets prescribed for treatment of disease should not be confused with diets which a clinically healthy person should and can eat.

In light of present knowledge, the best dietary recommendation for the average healthy adult is to eat moderately, choosing a variety of foods each day from these groups:

Milk group—two or more cups.
Cheese and ice cream can replace part of the milk.

Meat group—two or more servings.
Meat, fish, poultry, eggs, dry beans, peas, and nuts.

Vegetable-fruit group—four or more servings.
Include dark-green or yellow vegetables, citrus fruit or tomatoes, and other varieties.

Bread-cereals group—four or more servings.
Enriched, or whole, grain.

Excessive calorie and fat intake should be avoided by limiting use of added sugars and fats in food preparation and at table. Desired body weight should be maintained through regular exercise combined with moderate food intakes, to provide daily energy needs only.

Calorie and fat intake may have to be carefully restricted for persons with a family background of coronary heart disease, with obesity, high blood pressure, or high blood cholesterol levels, or with previous history of heart disease. This should be done only upon advice and supervision of a physician.

What the Dairy Industry Is Doing

The interest of the dairy industry in relationship of diet, particularly dietary fat, to coronary heart disease predates the general interest now in evidence. The entire industry has looked to National Dairy Council for leadership in this and other areas pertaining to nutrition and health. Financial assistance is provided by the American Dairy Association and all segments of the dairy industry. Through the cooperative efforts of national organizations, the dairy industry has sponsored research in this area for more than ten years, expanding the program as new evidence indicated need for additional basic investigation. Among other things, current studies include investigation of: *(1)* The effects of diet on serum lipid levels of human subjects in controlled dietary situations and among population groups; *(2)* the effects of moderate and low-fat reducing diets on the health of obese middle-aged men and women; *(3)* the metabolic functions of essential fatty acids and related nutrients, as revealed through animal and chemical experimentation and clinical work with human subjects.

To guide investment of research funds in this area, a series of research conferences has been sponored by National Dairy Council on behalf of the dairy industry, to determine how investigations ought to be directed to best provide knowledge of the role of fat in human health; and specifically to determine the relationship of diet to coronary heart disease.

Research findings have been widely disseminated to researchers, physicians, medically related professional groups, science writers, educators, and those dealing with directing information to the public. This has been done through distribution of reprints of scientific studies, interpretive reviews of research through *Dairy Council Digests* and *Nutrition News*, through advertisements in professional journals, through special articles such as this one, and through special materials.

For instance, a professional booklet and an industry leaflet on the subject have been prepared by National Dairy Council and should be available by September of 1957. In addition, newsworthy and reliable information is released to the press when deemed of value to the public, and assistance is given to editors and publishers by, providing them with regular releases and materials, counseling with writers regarding stories, and providing information and editing service on this subject. Information is also provided upon individual request.

The Dairywide Coordinating Committee on Nutrition Research has selected this problem as their major project for 1957. National Dairy Council serves as headquarters for this committee. Through the cooperation of the 14 member organizations of that Committee it is hoped that: *(1)* public relations can be maintained on a very sound level for the dairy industry with respect to this problem; *(2)* needed research can be supported by the dairy industry, to help solve this public health problem; and *(3)* evaluation can be made of any action which the dairy industry might advisedly take with respect to its products, should confirmed research findings indicate need for action on the part of the dairy industry.

ABSTRACTS OF PAPERS PRESENTED AT SOUTHERN DIVISION MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

BIRMINGHAM, ALABAMA, FEBRUARY 4-6, 1957

Effects of Water Used to Reconstitute Nonfat Dry Milk Solids. A. V. MOORE, Texas Agricultural Experiment Station, College Station.

The renneting time of reconstituted nonfat dry milks, simulating methods used in the manufacture of Cheddar cheese, ranged from 2 min., 55 sec., to 16 min., 37 sec., when 48 different waters and one source of instant-type nonfat dry milk solids were tested. When the waters were tested for conductivity, bicarbonate, carbonate, and total hardness, only total hardness was found to be indicative of renneting time. Longer renneting times were always associated with weak curd formation. Renneting times up to 7 min. resulted in curd that cooked satisfactorily.

The critical upper level of bicarbonate in waters was 300 p.p.m., in studies on the incidence of gas in curd made from synthetic waters and nonfat dry milk solids. The presence of $CaCl_2$ or NaCl up to 300 p.p.m., with and without bicarbonate, did not affect the gas-forming characteristics of the latter. Neither the starter nor the reconstituted milk contained gas-forming microorganisms.

Naturally hard waters, low in bicarbonate, are not improved by adjusting pH levels below 6.8. The curd is weaker in these waters. Commercially softened water (pH 8.3) produced a weak, gassy curd after adjustment to pH 6.0.

The Educational and Professional Status of Sanitarians. J. J. SHEURING, Univ. of Georgia, Athens.

The objectives of the study were to determine the educational, financial, marital, and professional status of milk and food sanitarians in the United States. The major objective was to determine the attitudes of sanitarians concerning their desire to obtain professional status through registration.

Approximately 4,000 questionnaires were sent to all members of the International Association of Milk and Food Sanitarians. Data were obtained concerning their *(1)* ages, *(2)* marital status, *(3)* salary ranges, *(4)* attitudes about their recommendations regarding the field of public health as a career, *(5)* educational training and experience, *(6)* present professional status, and *(7)* opinions about trying to establish professional status for sanitarians.

The following conclusions about sanitarians were made from the study: *(1)* Most of them are well-trained, *(2)* their financial status needs considerable improvement, *(3)* a great majority of them are eager to secure professional status, and *(4)* in general, they recommend the fields of public health and sanitation as a career.

Alfalfa, Clover, and Oat Pasture for Dairy Heifers. S. P. MARSHALL, Florida Agricultural Experiment Station, Gainesville.

A pasture mixture of Hairy Peruvian alfalfa, Kenland red clover, California burr clover, white clover and oats, seeded on October 6, was grazed from December 1 through September 20. The pasture was grazed a total of ten rotations during this period. A portable-type electric fence was used to restrict grazing to narrow bands of forage, and the animals to narrow strips across the field during each rotation.

Young dairy heifers grazing this pasture without receiving supplemental feed made average daily gains of 1.32 lb., which was 145% of the normal growth rate for animals of their ages. Gains per acre of pasture grazed averaged 755 lb. The animals obtained from the pasture an average of 5,227 lb. of total digestible nutrients per acre, which is equivalent to that in 5.2 lb. of alfalfa hay, all analyses. Cost of pasture production was calculated to be $72 per acre.

Nitrogen Retention in Dairy Calves. J. L. GRIFFITH, M. RONNING, and W. D. GALLUP, Dairy Department, Oklahoma A & M College, Stillwater.

Eight young dairy calves were removed from their dams at two days of age and placed on rations furnishing protein and T.D.N. in accordance with Morrison's 1948 minimum recommended allowances. Six metabolism trials with each calf were planned to begin at 9, 30, 51, 72, 93, and 114 days of age. Each calf was maintained at constant feed intake in an individual holding stall for at least four days prior to the preliminary period.

Growth curves were determined for each calf by plotting live weight measurements taken while the calf was in the holding stall prior to each trial. The live weight gain was estimated by determining the slope of the growth curve on the center day of the collection period.

The average apparent digestion coefficients for nitrogen were 92 and 95 for whole milk, 86 and 77 for the milk and grain ration, and 65 and 59 for the grain and hay ration.

Nitrogen retention averaged 16.2 g/1 lb. live weight gain at ten days of age, as compared to 6.59 g. at 100 days of age. Nitrogen retention in this study appeared to be in proportion to live weight to the 0.79 power.

Heritability Estimates of Type Characteristics in Guernsey Cattle. E. R. BEROUSEK, Dairy Department, Oklahoma A & M College, Stillwater.

The data used in obtaining the results of this report were taken from the files of the American Guernsey Cattle Club. All herds throughout the United States that were officially classified from 1947-1955 and on H.I.R. test were included. This report is based on a total of 3,202 cows found in 239 different herds. They were selected on an intra-sire-intra-herd basis and represent 1,981 daughter-dam pairs and 511 sires × herds subgroups.

Type information was based on the first available record. The heritability estimates calculated by doubling the intra-sire-intra-herd correlations and regressions of daughter on dam, respectively, were as follows: General appearance .24 - .24; shoulders .22 - .20; rump .28 - .28; dairy character .16 - 16; body capacity .28 - .28; udder .24 - .24; fore udder .24 - .22; rear udder .26 - .26; teat placement .32 - .30, over-all rating .28 - .28.

Feet and legs were omitted from the study, because the method of recording these type classification ratings was not consistent throughout the entire period.

Control of Bacterial Spoilage in Cottage Cheese. H. C. OLSON and O. D. BALL, Dairy Department, Oklahoma A & M College, Stillwater.

In cottage cheese at the usual pH levels, a concentration of 0.04% sorbic acid was generally effective in preventing spoilage due to yeasts and molds, and a concentration of 6 p.p.m. of aureomycin was generally effective in controlling bacterial spoilage. A combination of the two inhibitors was generally effective in preventing spoilage in cheese inoculated with the bacterial species and with molds. However, the inhibitors were generally ineffective when the pH level was above that normally found in satisfactory cheese. Spoilage was much more rapid and extensive at 55 than at 45° F.

A combination of the two inhibitors was added to several samples of commercial cottage cheese and the keeping qualities at 45 and at 55° F. determined. With several of the samples, the inhibitors greatly improved the keeping quality, but in several other instances the inhibitors had no significant effect on the keeping quality. Differences in pH and in degree of contamination among the samples may largely account for failure of the inhibitors to improve the keeping quality of the samples that spoiled rapidly.

Methods for Testing Cottage Cheese. H. C. OLSON, Dairy Department, Oklahoma A & M College, Stillwater.

Various modifications of the Babcock Test, including the regular Babcock test, Minnesota test, Illinois test, Pennsylvania test, and others, were run on samples of cottage cheese and the results compared with the Mojonnier test as a standard for comparison. Some of the tests were satisfactory from the standpoint of simplicity, ease of performance, and clarity of fat column, but were rejected because they did not agree closely with the Monjonnier tests. Two tests which appeared to be the most satisfactory were the "acid-QAC" test and the Minnesota test. The "acid-QAC" test involves the use of sulfuric acid with 9 ml. of alkyldimethylbenzyl ammonium chloride added per liter to digest the curd. The curd is digested in a small flask and then transferred to a Babcock test bottle. The Minnesota test involves the use of Minnesota reagent and a Waring Blendor to form a creamy mixture, which is weighed into Babcock test bottles and the Minnesota test completed as for milk. Both these tests gave satisfactory results when compared to the Mojonnier test.

Length of Estrous Cycle of Dairy Cows Following Infertile Service. O. R. KEIRN and S. D. MUSGRAVE, Dairy Department, Oklahoma A & M College, Stillwater.

The literature indicates the normal length of the estrous cycle for dairy cows to be from 18 to 24 days, with approximately 84% of the observations falling within this range.

It was the purpose of this report to obtain data concerning the estrous cycle of cows that failed to conceive to first service. One hundred and seven Holstein, 81 Guernsey, 113 Ayrshire, and 93 Jersey cows had 477, 379, 460, and 464 such estruses, respectively. These cows averaged 2.9 estruses following infertile service per gestation. Also, they averaged 4.5 estruses following infertile service while in the herd. These estrous cycles averaged 43.17, 40.17, 39.60, and 43.35 days in length, respectively, for the Holstein, Guernsey, Ayrshire, and Jersey cows, with an over-all average length of 41.66 days. Only 41% of such estrous cycles fell in the range of 18 to 24 days. There were 65, 734, 244, 216, 172, 103, 54, 53, and 139 cycles within the ranges of 2-17, 18-24, 25-38, 39-44, 45-59, 60-67, 68-77, 78-86, and 87-324 days, respectively. All data relative to known abortions were removed from these data.

Effect of Containers on Motility of Deep-Frozen Bull Spermatozoa. S. D. MUSGRAVE and A. L. HEATH, Dairy Department, Oklahoma A & M College, Stillwater.

There have been numerous examples in field use of frozen semen, in which semen stored

in glass exhibited greater motility when thawed than did semen from the same ejaculate that was frozen in plastic containers.

Two short-term trials were conducted to define the problem. In the first trial, survival on thawing was poor because a sulfanilamide diluter was inadvertently used. This concerned 13 ejaculates from eight bulls. Per cent motility was higher for semen in plastic immediately after freezing, but semen frozen in glass had a slight advantage in storage for from one to two weeks and at 90 days. A record trial involving 30 ejaculates from ten bulls yielded acceptable survival rates after freezing. Split-ejaculates were again used. There was no difference due to sealing effect of either container before freezing. Semen stored in glass had a slight advantage when observed immediately after freezing, a definite advantage after two weeks of storage ($P < 0.05$) and 90 days of storage ($P < 0.01$) in terms of per cent motile sperm. This was true for simulated shipment. The multiple range test for individual ejaculates and analysis of variance by groups led to the same interpretation of results.

Effects of Protein Levels of Starters with and Without Aureomycin Supplementation on Growth and Metabolism of Young Dairy Calves. J. P. Everett, Jr., L. D. Brown, C. A. Lassiter, D. R. Jacobson, and J. W. Rust, Univ. of Kentucky, Lexington.

In an experiment utilizing 60, two-day old Holstein and Jersey calves and five starters ranging from 6.3 to 14.2% protein, the calf starter containing 14.2% protein was found to be adequate for normal growth (1.22 lb/day) of young dairy calves raised on a limited milk-hay-starter system to 86 days of age. When aureomycin was included in the starter, above-normal gains, 1.25 and 1.32 lb/day, were made by calves on 12.2 and 14.2% protein starters, rsepectively. Also, increases in average daily gains, height at withers, heart girth circumference, and starter consumption were obtained. Aureomycin had no significant effect on apparent digestibility of any of the nutrients in the ration, nitrogen retention, blood levels of plasma protein, urea, or nonprotein nitrogen; however, this antibiotic tended to lower slightly the blood levels of urea and nonprotein nitrogen. Aureomycin gave a slight but nonsignificant depression in rectal temperatures. Calves on the 12.2 and 14.2% protein starters had significantly higher coefficients of apparent digestibility of crude protein, and the 14.2% protein groups had significantly greater nitrogen retention than did calves on the 6.3% protein starters.

Aureomycin (Chlortetracycline) Distribution in Milk. L. L. Rusoff, G. C. Lee, and E. J. Stone, Louisiana State Univ., Baton Rouge.

The degree of retention of aureomycin by certain constituents of milk, and the rate of elimination of the antibiotic in the milk after treatment for mastitis were studied.

Milk from both healthy and mastitic cows treated with aureomycin by mammary infusion was tested for antibiotic distribution by the pad-plate microbiological assay method. The concentration of the antibiotic in the milk following infusion dropped considerably after the first eight milkings, and only a trace remained at the end of ten days.

The antibiotic was concentrated in the skim-milk fraction. During the first four to five milkings the highest concentration was found in the filtrate remaining after removal of casein and albumen, the next highest in the casein, and lowest in the albumen. Subsequent milkings showed the highest concentration of the antibiotic to be in the casein, and lowest in the filtrate fraction. Constitutents of the milk from healthy cows contained two to three times more antibiotic than those in the milk of mastitic cows.

Response of Young Dairy Calves Fed a Simple vs. a Complex Starter with Different Hays. W. R. Murley, R. D. Mochrie, J. R. Edwards, and B. F. Hollon, N. C. State College, Raleigh; Mountain Research Station, Waynesville; and Coastal Plain Research Station, Willard, N. C.

Forty-five Jersey calves were randomly assigned at four days of age to receive either a complex or a simple starter limited to 3 lb. daily. Whole milk was fed through 35 days of age and various kinds of hay were fed ad lib. from seven days through 112 days. Daily gains were 0.97 and 0.94 lb., for calves getting the complex and simple starter, respectively. Starter consumption was 220 and 223 lb., hay intake was 136 and 115 lb., and the calculated TDN intake per pound of gain was 2.41 and 2.46 lb., respectively, for the complex and simple starter-fed calves.

Alfalfa, orchard-grass, coastal Bermuda, and oat hays were fed. The average daily gains for the calves getting the respective kinds of hay were 0.91, 0.87, 1.05, and 0.96 lb. The calves ate more of the alfalfa hay and less starter; whereas, the calves getting the other hay ate less hay and correspondingly more starter. The calculated TDN intake per lb. of gain was 2.61, 2.52, 2.22, and 2.41 lb., respectively, for the calves getting the four kinds of hay. The calves getting coastal Bermuda hay, irrespective of type of starter, were more efficient than the other groups.

Effects on Growth and Feed Consumption of Adding Brown Grease and Hydrogenated Cottonseed Oil to Calf Starters. W. J. Miller, J. L. Carmon, and H. L. Dalton, Univ. of Georgia, Athens.

A commercial-type basal starter was used

and the other four starters contained the basal plus the following: *(a)* 10% brown grease (stabilized), *(b)* 10% hydrogenated cottonseed oil, *(c)* 5% brown grease and 5% hydrogenated cottonseed oil, and *(d)* 10% brown grease and 10% hydrogenated cottonseed oil. All the starters contained 18% protein. In a growth study, seven replications of dairy calves were fed the starters ad libitum for 10 wk. Those fed the basal starter gained significantly faster and consumed significantly more starter than any of the others. There were no significant differences among any of the groups fed 10% added fats. Those fed starters containing 10% added fats gained significantly more weight and ate significantly more than those fed 20% added fats. The average daily gains of those fed the basal and the other four starters as listed above were 1.23, 0.83, 0.95, 0.88, and 0.63 lb. Some of the calves fed the starter with 20% added fats were chronically bloated during the last few weeks of the experiment.

In another experiment, ten calves were given all five starters in a cafeteria study. The basal starter was the decisive choice of nine. One calf ate significantly more of the starter containing 10% brown grease.

Influence of Feed and Environment on the Consumption of Milk. I. I. PETERS, R. E. LEIGHTON, and C. A. MULAY, Texas Agricultural Experiment Station, College Station.

The following information was obtained during an 8-wk., hot-weather, single-reversal trial using two lots of ten cows each, five Holsteins and five Jerseys. The high-fiber Ration B contained 63% more crude fiber and 14% more dry matter than did the low-fiber Ration C.

Individual cow's milk was examined weekly for conductivity at 0 and 37° C., freezing point, % total solids, % fat, % solids-not-fat, and % lactose.

Weekly averages of all cow's milk were significantly higher in % total solids and % fat when cows received Ration B. In addition to the above, individual cow averages were significantly higher in conductivity values at 37° C. while on Ration B.

With the Holstein group alone, Ration B resulted only in significantly higher % solids-not-fat; whereas, with the Jersey group alone Ration B resulted in significantly higher % fat and in significantly higher conductivity at 0 and 37° C.

When the cows were fed Ration C, milk production was 2.3% higher, body temperatures averaged 0.34° F. lower, and the respiration and pulse rates were six and three per minute slower than when fed on Ration B.

Maintaining Milk Production in Hot Weather by Adjusting Fiber Levels in the Ration. I. W. RUPEL and R. E. LEIGHTON, Texas Agricultural Experiment Station, College Station.

A second year's work was completed on the effects of fiber content of the ration on milk production during hot weather. On the higher fiber diet, the cows consumed an average of 1.1 lb. alfalfa hay and 2.4 lb. sorghum silage per 100 lb. body weight, supplemented by a grain mixture containing 10.4% fiber. On the low-fiber diet, the cows were limited to 0.75 lb. alfalfa hay and 1.5 lb. silage per 100 lb. body weight. Their grain mixture contained only 4.1% fiber. In a reversal trial, the experimental cows in 1955 produced an average of 3.2 lb. more milk per cow daily and showed a 0.3 degree F. lower body temperature, a 6.5 per minute lower respiration rate, and 4.5 per minute lower pulse rate when they received the low-fiber ration. During 1956, the cows averaged 0.96 lb. more milk per day, showed a body temperature of 0.37 degree F. lower, a respiration rate of 6.3 per minute lower, and a pulse rate of 3.6 per minute lower than were observed on the high-fiber ration. These average differences, paired by daily lot averages for the two groups, were highly significant.

Effects of Feeding Thyroxine or Thyroprotein to Identical-Twin Dairy Cows for Successive Lactations. E. W. SWANSON, S. A. HINTON, and C. E. WYLIE, Dairy Department, Univ. of Tennessee, Knoxville.

Five pairs of identical-twin Jersey and Guernsey cows were assigned at random, prior to their first lactation, to either a normal or treated group. In each lactation the treated twins have been fed iodinated casein (thyroactive protein) or l-thyroxine from about the 20th wk. of lactation to the end. All pairs have completed two lactation comparisons. Comparisons in the third lactation have been made for three pairs, and two pairs are now in their fourth lactations. Continuous records have been made of milk and fat production, body weights, heights, and heart girth. Pairmates were fed the same, except as milk production was increased due to thyroxine feeding, when the treated cows were given extra grain. In the first lactation, the average yield of FCM prior to treatment was 3,632 and 3,709 lb. from control and treated groups. After treatment, FCM production was 2,181 and 2,726 lb., respectively. In the second lactation, pretreatment yields of FCM were 4,523 and 4,533 lb. for treated and control groups. After treatment they produced 2,333 lb. and 2,449 lb., respectively. The twin pairs have remained nearly identical in height. The thyroxine-fed cows averaged 22 lb. lighter than the controls at the end of the second lactation. Two of the three third lactation pairs also produced comparably, but the thyroxine-fed cow of the third pair produced lower than its mate. These data indicate that thyroxine feeding for two successive lactations has had no apparent harmful effects upon these cows.

Reproduction and calf health have been normal in both groups.

Some Mathematical Relationships Between Various Components of Mixed-Herd Milk. J. B. MICKLE, E. R. EGERMEIER, F. A. GRAYBILL, S. D. MUSGRAVE, and R. MACVICAR, Departments of Dairying, Mathematics, and Agricultural Chemistry, Oklahoma A & M College, Stillwater.

In this study, an attempt was made to evaluate some of the calculations used in the creamery to predict the amounts of protein, lactose, or ash present in mixed-herd milk on the basis of the fat content of that milk. Daily samples of mixed-herd milk representing the night and morning milkings of the Oklahoma A & M College herd were collected six days per week, every other week, from November, 1955, to November, 1956. These samples were analyzed for their fat, lactose, ash, and total nitrogen contents.

Monthly averages calculated from these data showed the seasonal trends indicated in the literature. However, in this work, the day-to-day and week-to-week variations of the various milk components were often as large as were the seasonal differences.

Regression equations calculated from these data were: *(1)* % protein = 1.974 + 0.389 F., *(2)* % lactose = 5.747 − 0.235 F., and *(3)* % ash = 0.508 + 0.054 F. The 95% confidence limits for these equations were *(1)* ± 0.17%, *(2)* ± 0.43%, and *(3)* ± 0.03%.

Relative Value of Selected Covers for a Trench Silo. J. T. MILES, J. R. OWEN, and J. W. LUSK, Mississippi State College, State College.

Corn silage was packed continuously with a tractor during filling, and experimental covers were put on in strips across a 20 ft.-wide trench silo. Measurements of loss were determined by reference to a series of steel stakes driven 3 in. below the surface of the silage.

Average total loss of silage in inches and pounds for the respective covers in a 2 × 20 ft. area were: 11.7 in. and 1,317 lb. for the uncovered controls; 10.2 in. and 1,154 lb. for a three-inch layer of oat straw covered with dirt; 10.0 in. and 1,073 lb. for 3 in. of agricultural limestone; 3.8 in. and 429 lb. for lightweight roofing felt covered with from 2 to 3 in. of sawdust; 0.7 in. and 106 lb. for 6-gauge clear polyethylene plastic and from 2 to 3 in. of sawdust. Results with plastic used a second year, and under the lap of two pieces of plastic covered with sawdust, were essentially the same as for new plastic. The same plastic with only the edges covered was unsatisfactory, due to cracks caused by exposure to climate.

A Comparison of Systems of Grazing a Summer Annual Crop. J. R. OWEN, J. T. MILES, and W. C. COWSERT, Mississippi State College, State College.

Three groups of ten cows each were used to compare rotational grazing, strip grazing, and green feeding of Tift sudan grass. Three paddocks of two acres each provided pasture for the rotational group. During a 68-day period, the entire six acres of rotational pasture were used, and returned 111 cow-days' grazing per acre. Approximately 4.1 acres returned 149 cow-days' grazing per acre in the strip-grazing area, and 3.4 acres provided 180 cow-days' supply of forage per acre when harvested and fed green. The average daily production of F.C.M. and per cent decline from a 31-day base period were as follows: rotational group, 28.0 lb. and 23.1%; strip grazing, 26.6 lb. and 26.2%, and green feeding, 29.0 lb. and 20.2%. These differences were not significant. Average gains in body weight per cow were 10.7 lb. for the rotation group, 7.4 lb. for strip grazing, and 30.5 lb. for green feeding. At the conclusion of the test period, the forage remaining in the rotational pastures appeared more healthy and vigorous than that used by strip grazing or green feeding; however, further measurement of production was prevented by severe army-worm damage.

Comparisons of Various Media for Culturing Bovine Testicular Tissues. M. A. BROWN, Texas Agricultural Experiment Station, College Station.

Nutrient media used to study animal tissues of other species in culture were tested, to discover a medium which would best support growth of bovine testicular tissue in vitro. Testicular material was obtained either by biopsy or at slaughter. The roller-tube method of culture was used throughout the study, and a roller-tube apparatus designed for this study as a modification of earlier designs was found to be satisfactory in all respects. The tissues were cultured at a temperature of 32° C.

Under the conditions of this study, a medium of bovine serum, dilute embryo extract, and balanced salt solution was found to maintain growth of male bovine germinal epithelium for as long as 40 days in culture. Tissues nourished by this fluid released actual concentrations of spermatozoa as high as 45 per cm. of fluid in a four-day period. These concentrations were greater than those observed for the other treatments.

It appears that the age of bulls at the time testicular tissues are excised may influence the livability of the tissues in vitro.

Cryoscopic Estimations of Added Water in Milk. T. R. FREEMAN, Kentucky Agricultural Experiment Station, Lexington.

Formulas used for calculating added water from F. P. determinations were:

$$W = \frac{0.550 - T}{0.550} \times 100 \quad (1)$$

In which W = % added to water
T = F. P. in degree Centigrade below zero

$$W = \frac{T - T'}{T} \times 100 \quad (2)$$

In which W = % added to water
T = F. P. of "unwatered" sample in degrees Centigrade below zero
T = F. P. of "watered" sample in degrees Centigrade below zero.

Both formulas gave a calculated amount of added water in excess of the actual amount. A part of this "error" may be explained by the fact that the addition of water to milk raises the freezing-point of the milk only to the extent that the extraneous water dilutes the true solution of the milk. It is believed that the remainder of the error results from the fact that the A.O.A.C. standard for the freezing-point of pure milk is slightly low. (A statistical analysis of 546 individual cow samples analyzed at this station suggests that the upper freezing-point limit for a 50-cow composite sample should be — 0.544° C.)

A modification of the A.O.A.C. formula, to accommodate the above two principles, results in the following formula:

$$W = \frac{(0.544 - T)\ 0.92}{0.544} \times 100 \quad (3)$$

In which W = % added water
T = F. P. of sample in degrees Centigrade below zero.

Use of the "Vacu-Therm" Pasteurizer for Milk Flavor Control. J. T. Lazar, Jr., and W. R. Bellamy, South Carolina Agricultural Experiment Station, Clemson.

Onion-flavored milk was treated under vacuum in the DeLaval Vacu-Therm Pasteurizer. The milk was pasteurized at 175° F., at which temperature it entered the vacuum chamber, where it flashed immediately under the reduced pressure. The flashing points of the milk were adjusted to 140, 150, and 160° F. by regulating the levels of vacuum. As a control, normal-flavored milk was treated in a similar manner, in order to make comparisons of the two milks at each temperature treatment. Sixty-four persons were asked to compare the two samples of each treatment, and to indicate if there were any differences.

The onion flavor was detected in the raw sample by 60 of the 64 judges. When the milk was subjected to the vacuum treatments, fewer of the judges detected the onion flavor. As the level of vacuum increased, the number of judges who detected the onion flavor decreased. With the maximum treatment, only nine judges identified the difference in flavor as being onion. The nine judges who detected a difference in the samples did not express a strong dislike for the onion-milk treated at the maximum treatment.

The results of this experiment indicate that the DeLaval Vacu-Therm Pasteurizer is effective in removing onion flavors from milk.

Comparison of Two Citrus Supplements on Silage Consumption, Milk Production, and Weight Changes. H. L. Dalton, W. J. Miller, and J. D. Donker, Univ. of Georgia, Athens.

Silages made from Abruzzie Rye and Crimson Clover were used to compare citrus mean and citrolas upon milk production, silage consumption, and weight changes in dairy cows. Citrus meal was added to one of the silages at the rate of 250 lb. per ton when ensiled. Citrolas was added to the other silage when fed at a rate of 240 lb. per ton. The experiment was a single reversal with 28-day feeding periods, with the first seven days being an adjustment period. Concentrate was fed at the rate of 1 lb. to each 6 lb. of 4% F.C.M. produced in the week prior to the first adjustment period. There were no significant differences in 4% F.C.M., fat %, or gains between the cows consuming the two silages. Cows fed the silage with citrolas consumed significantly more silage and highly significantly more silage dry matter. Average daily consumption of the cows fed the two silages was 64.1 vs. 59.6 lb. and 16.4 vs. 13.6 lb. of silage dry matter per cow. In the one-week change-over period, the cows switched from citrus-meal silage to silage with citrolas gained 11.6 lb. per cow more than those switched in the opposite direction.

Effects of Seven Nongenetic Factors on Butterfat Production in Holsteins. N. R. Thompson, P. M. Reaves, C. Y. Kramer, and G. C. Graf, Virginia Agricultural Experiment Station, Blacksburg.

A total of 193 lactation records on 74 Holstein cows were analyzed for the joint effects of seven nongenetic factors on butterfat yield. A least-squares form of analysis was used. The solution gave linear partial regressions of butterfat yield on year of calving (Y), previous calving interval (K) in months, days milked (L), previous dry period (D) in days, and days of gestation (G) during the lactation period of — 7.1, 4.1, 1.2, 0.4, and 0.06 lb., respectively. The effects of calendar month of calving (M) and age at calving (A) were

estimated (in the same solution) as curvilinear functions. For (M), linear and quadratic terms were − 9.2 and 0.7 lb., respectively; for (A), 5.2 and − 0.02. The partial regressions of (L) and (A) were significant at the 1% probability level; the other regressions were not significantly different from zero. Variance components were estimated as 256,618 for (L), 32,793 for (A), and only 781 for the other five variables together.

Response of Growing Dairy Heifers to Frequency of Feeding. W. A. HARDISON, A. H. RAKES, R. W. ENGEL, and G. C. GRAF, Virginia Agricultural Experiment Station, Blacksburg.

Twelve dairy heifers, ranging in size from approximately 300 to 500 lb., were used in a single-reversal trial to study the weight-gain response of growing animals to frequency of feeding. The animals were fed mixed alfalfa–orchard grass hay of medium quality, which had been run through a silage-chopper to facilitate feeding. The animals were brought up to maximum feed intake during a 21-day preliminary period. This level of feed intake was maintained during the two, 50-day experimental periods which followed. Treatment 1 consisted of giving the animals their daily feed allowance in two feedings daily (8 A.M. and 5 P.M.), and Treatment 2 consisted of giving the animals exactly the same quantity of feed divided into small portions and fed at hourly intervals between 8 A.M. and 5 P.M. The animals were weighed on three consecutive days, at the begining and end of each experimental period, and at 10-day intervals. The total body weight gains made by the animals fed two and ten times per day were 294 and 565 lb., respectively, for the 100-day feeding period. The difference in weight gain was highly significant.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL HEALTH

518. Isolation of Vibrio fetus from bulls. W. N. PLASTRIDGE, E. C. WALKER, L. F. WILLIAMS, E. F. STULA, AND E. M. KIGGINS, Storrs Agr. Expt. Sta., Storrs, Conn. Am. J. Vet. Research, **18**, 68: 575. 1957.

Samples of semen and preputial washings from 149 bulls used in four artificial breeding centers were examined for the presence of *Vibrio fetus*. Blood agar plates incubated in an atmosphere of 5% O_2, 10% CO_2, and 85% N_2 were more efficient in detecting *V. fetus* than other cultural methods. Colonies resembling *V. fetus* were subcultured in thiol medium, tested for catalase activity, and examined microscopically. The incidence of *V. fetus* infected bulls was 41%. Of these, 39% were detected in one test, 67% by two tests, and 95% by three tests. Of 544 semen camples examined, 27% yielded *V. fetus*. Forty-nine % of the *V. fetus* cultures isolated were not agglutinated by three types of antisera. Only 10% of the positive bulls tested gave positive blood serum agglutination tests, showing that *V. fetus* has little pathogenicity for the bull and exists primarily as a saprophyte in the genitourinary tract of carrier bulls. The danger of transmission of *V. fetus* by ordinary methods of semen collection is stressed. The average concentration of *V. fetus* in positive semen samples was 800 per ml.

E. W. Swanson

519. Observations on experimental bovine Vibrio fetus infection. H. BLOBEL, J. SIMON, AND S. H. MCNUTT, Wis. Agr. Expt. Sta., Madison. Am. J. Vet. Research, **18**, 68: 579. 1957.

Three groups of seven heifers each were, respectively, *(a)* inoculated with *Vibrio fetus* intravaginally, *(b)* inoculated intrauterinely in estrus, and *(c)* used as nontreated controls. Blood and vaginal mucus agglutination reactions to five strains of *V. fetus* were recorded biweekly. Five of the Group A heifers developed vaginal mucus agglutinins, and *V. fetus* was consistently isolated from vaginal mucus of one. Five of the Group B heifers also developed vaginal mucus agglutinins, but none of the control heifers did. In Groups A and B, the heifers with vaginal mucus agglutinins required more services for conception and had more irregular and prolonged estrous cycles than did the normal heifers. Low blood titers developed in the control as well as the treated heifers, and were not correlated with the vaginal mucus titers. It was concluded that the disease is primarily localized in the genital tract of the cow and that the vaginal mucus agglutination test is the most valuable diagnostic test.

E. W. Swanson

520. Studies on the pharmacology of sulfaquinoxaline in dairy cattle. C. M. STOWE, D. PALLESSEN, AND W. HARTMAN, Minn. Agr. Expt. Sta., St. Paul. Am. J. Vet. Research, **18**, 68: 511. 1957.

Sulfaquinoxaline, which is used widely for treatment of poultry coccidiosis, was studied for its possible application in calves and cows. It failed to maintain satisfactory blood levels more than a few hours after intravenous or oral dosage. Toxic signs, ataxia, and paralysis also developed in calves. Kidney damages were noted in calves, and crystalluria in cows. These observations indicate that this sulfonamide would not be satisfactory for use in dairy cattle.

E. W. Swanson

BOOK REVIEWS

521. Danish Dairying. EINAR O. PETERSEN. Translated by G. H. Wilster. Dairy Publishing House, Copenhagen, Denmark.

This book provides enjoyable reading, as well as detailed information, concerning the status of dairying in Denmark.

Denmark has no natural resources other than her soil; thus, agriculture is her principal industry, with dairying playing a prominent role. The Danes have developed and take pride in their own breeds of cattle. The Red Danish and the Black and White Danish cows represent from 85 to 90% of the dairy animals in the country. Bovine tuberculosis was eliminated in 1952 and a concentrated effort will eliminate Bang's Disease in the near future.

Dairy plants are comparatively small, but are well equipped with machines, mostly of Danish construction. Production and marketing cooperatives are common and successful, but privately owned plants are also important.

Butter and cheese are the principal dairy products made. Cheese manufacture has increased tremendously during the past 10 yr., making Denmark the third largest cheese exporter in the world. The by-products, skimmilk and cheese whey, are utilized in the production of bacon.

Market milk and ice cream account for 12 to 13% of the milk production.

The book is illustrated by numerous excellent photographs of Danish farms and plants.

P. Hansen and J. Tobias

BUTTER

522. Bepaling van enkele kenmerkgetallen van Belgische melkerjboter (Determination of some typical values of Belgian dairy butter). M. Naudts and A. De Vleeschauwer. Overdruik Uit Mededelingen van de Landbouwhogeschool en de Opzoekingstations van de Staat te Gent, **21**, 2: 227. 1956.

During the years 1953-1954 the authors determined the Reichert-Meissl and Polenske values, the butyric acid values according to Grossfeld, and the refractive index on butter from 208 dairies. Progress and the variations of the values are given and discussed. The Reichert-Meissl value seems especially useful for the detection of adulteration of butter, provided the season and place of production are known and the necessary reference material is available. It is also advisable to determine the refractive index. F. W. Bennett

523. The butter situation. R. Fifer, American Butter Institute, Chicago, Ill. S. Dairy Prod. J., **61**, 1: 27. 1957.

Butter consumption in the United States continues to increase. Butter consumption in 1956 was almost double the per capita civilian consumption during rationing, in World War II. Price-support surplus stocks have been completely utilized. Interest in butter cookery is increasing, and advertising and promotion efforts are greater. The butter industry is still a billion-dollar industry and is the important balance-wheel of the whole dairy industry.

F. W. Bennett

524. Butter slicing and dispensing device. E. E. Esko. U. S. Patent 2,799,931. 5 claims. July 23, 1957. Offic. Gaz. U. S. Pat. Office, **720**, 4:691. 1957.

Pats of butter are sliced from a quarter-pound print by a knife equipped with a pivoted handle. R. Whitaker

CHEESE

525. Manufacture of cheese slices. B. T. Hensgen and J. C. Vanden Bosch (assignors to Swift and Co.). U. S. Patent 2,799,586. 10 claims. July 16, 1957. Offic. Gaz. U. S. Pat. Office, **720**, 3:598. 1957.

Process cheese is molded in a series of thin frames and, after setting the pieces in the size and thickness of individual slices of cheese, are pressed out of the frames and stacked to form a package of solid cheese.

R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

526. Het gebruik van wei en afgeroomde melk bij de biosynthese van vitamine B_2 door Eremothecium ashbyii. Invloed van de hoeveelheid entcultuur (The use of serum and skimmilk for the biosynthesis of vitamin B_2 with Eremothecium ashbyii. Influence of the quantity of bacterial culture). H. Hendricks and A. De Vleeschauwer. Overdruik Uit Mededelingen van de Landbouwhogeschool en de Opzoekingstations van de Staat te Gent, **21**, 1: 67. 1956.

It appears from authors' results that very small amounts of inoculum may be added to culture media without influencing the production of riboflavin. The numbers of the microorganisms in the inocula used varied from a few to several thousand. Mycelium or spores may be trapped in the foam, greatly reducing riboflavin production. Foam formation may be prevented by adding antifoam compounds such as soy bean oil. Very active cultures are more important than the number of bacteria. The highest riboflavin production was obtained from a medium of one-half whey and one-half skimmilk plus 5% saccharose. Fermentation tests were at from 26 to 28° C. for 6-8 days.

F. W. Bennett

527. Pudding composition and process of producing the same. A. S. Clausi (assignor to General Foods Corp.). U. S. Patent 2,801,924. 27 claims. Aug. 6, 1957. Offic. Gaz. U. S. Pat. Office, **721**, 1:134. 1957.

A pudding made from milk by reacting the casein and albumin with an alkali pyro- and orthophosphate. R. Whitaker

528. Compositions for preparing puddings. L. S. Stoloff (assignor to Seaplant Chemical Corp.). U. S. Patent 2,801,923. 18 claims. Aug. 6, 1957. Offic. Gaz. U. S. Pat. Office, **721**, 1:134. 1947.

Cold milk is mixed with a mixture of an extract of a marine plant and salts of calcium and magnesium phosphate, carbonate, or sulphate to form a pudding. R. Whitaker

DAIRY BACTERIOLOGY

529. Rumen microbiology. Characteristics of free rumen cellulases. J. W. GILL AND K. W. KING, Dept. of Biochem. and Nutr., Va. Agr. Expt. Sta., Blacksburg. J. Agr. and Food Chem., **5**: 363. 1957.

By using the viscometric assay for cellulase with a carboxymethyl-cellulose substrate, the presence of soluble cellulases in rumen ingesta has been demonstrated. At least three enzymes appear to be involved. These enzymes are relatively thermostable and insensitive to oxygen, and have a pH optimum of about 6.5. Some samples of rumen ingesta contain a cellulase inhibitor. In other samples, no evidence of inhibitor is seen, but an activator is present.

S. Patton

530. An evaluation of milk quality as influenced by daily vs. every-other-day pickup of milk cooled in farm milk tanks. H. V. ATHERTON AND A. BRADFORD, Dept. of Dairy Husbandry, Univ. of Vermont, Burlington. J. Milk and Food Technol., **20**: 223. 1957.

Milk samples obtained from each milking of an EOD pickup system were comparable to fresh milk held below 40° F. The standard plate count, laboratory pasteurized and psychrophilic counts were almost identical. Protein stability was slightly altered, but this did not appear to be caused by EOD pickup. Where recommended sanitary practices were observed on the farm, the EOD system maintained a satisfactory raw milk during a normal period of transportation or holding prior to pasteurization.

H. H. Weiser

531. A bacteriological survey of commercially frozen beef, poultry, and tuna pies. W. LITSKY, I. S. FAGERSON, AND C. R. FELLERS, Dept. of Bacteriol. and Food Technol., Univ. of Mass., Amherst. J. Milk and Food Technol., **20**: 216. 1957.

A tentative bacteriological standard of 100,000 organisms per gram for frozen beef, poultry, and tuna is proposed. A survey of retail stores handling these products showed the presence of enterococci, coliform, and *Salmonella* types of organisms in the total bacterial count.

H. H. Weiser

DAIRY CHEMISTRY

532. Milk proteins. Quantitative ionophoretic determination of some whey proteins in skimmilk. A. LEVITON, Eastern Utilization Research Branch, Agr. Research Serv., USDA, Washington, D. C. J. Agr. Food Chem., **5**: 532. 1957.

The quantitative determination of whey proteins is a matter of considerable interest in dairy technology. Studies with α-lactalbumin and β-lactoglobulin, singly and in mixtures, have shown that recovery is quantitative if a precoat of β-lactoglobulin is applied ionophoretically. This technique, applied to whey, yields quantitative values for the chief protein components. Streaking of milk samples 1 in. from the apex, toward the cathode, permits the easy resolution of β-lactoglobulin in milk, and its subsequent quantitative determination.

S. Patton

533. Silage analysis. Determination of organic acids in silage. H. G. WISEMAN AND H. M. IRVIN, Dairy Husbandry Research Branch, USDA, Beltsville, Md. J. Agr. Food Chem., **5**: 213. 1957.

Celite columns with an internal indicator have been developed for the quantitative separation of silage acids ranging from butyric to succinic. The aqueous internal phase employs minimal amounts of sulfuric acid to prevent retention of organic acids; sugar is added to the phase to increase elution resistance. Aqueous samples, 2 ml. or less, are added directly to a dry column cap. Eluting solvents are mixtures of acetone with Skellysolve B. The method is designed to eliminate steam distillation, or ether extraction of acids, and mechanical equipment for collection of fractions. Single zone collections make possible a reduced number of titrations with increased accuracy. The column permits fairly wide separations of lactic and succinic acids, often difficult on silicic acid columns.

S. Patton

534. Carotene assay. Determination of carotene in silages and forages. H. G. WISEMAN, H. M. IRVIN, AND L. A. MOORE, Dairy Husbandry Research Branch, USDA, Beltsville, Md. J. Food Chem., **5**: 134. 1957.

A rapid chromatographic method for determination of carotene in silages and forages is described. Necessary conditions for separation of carotene from impurities on magnesium oxide–Celite columns, in the presence of small amounts of alcohol, have been established. The analysis combines the advantages of splash-free blending extraction afforded by alcohol–Skellysolve B foaming mixtures and the elimination of epiphasic washing to remove alcohol. Additional advantages include faster and more compact elutions gained by the presence of alcohol on the column. Direct collections in smaller volumes eliminate concentration and transfer operations.

S. Patton

535. Chromic oxide measurement. Improved determination of chromic oxide in cow feed and feces. F. T. KIMURA AND V. L. MILLER, Western Wash. Expt. Sta., State Coll. of Wash., Puyallup. J. Agr. Food Chem., **5**: 216. 1957.

Chromic oxide is widely used as a reference substance in nutrition investigations of domestic animals. An analytical method that is simple, specific, rapid, and requires only common laboratory equipment, may be used to determine chromic oxide in feeds and feces in the amounts commonly used in digestibility experiments with dairy cattle.

S. Patton

DAIRY ENGINEERING

536. A new approach to ice cream production and engineering planning. E. L. FORSTER, Cherry-Burrell Corp., Cedar Rapids, Iowa. Ice Cream Trade J., **53**, 6:62. 1957.

The objectives of production planning are to reduce operating costs, reduce product losses, improve quality, increase sales opportunities, and relieve inventory problems in the hardening-room. The data necessary to make a production analysis include actual and anticipated production figures, complete list of equipment available for freezing and packaging, and the number of people used for each specific class of product.

Visual planning charts can be prepared on squared paper 11 in. by 16.5 in. Freezers are listed down the left-hand side, operating hours are shown vertically across the page; each operation performed by each freezer is recorded on the squared paper. A schedule can be made for an actual peak month, on the basis of present scheduling methods. Things to strive for are minimum crew for each operation, freezer capacity to balance optimum operating capacity, operations that can be run simultaneously, and those which should be run at separate times. Avoid scheduling operations when crews can work faster than the freezer capacity available.

Attainable operating rates should be set by the plant superintendent and special attention should be paid to the operations. The proposed schedule should be checked against hardening-room space available and rate at which product can be handled. This chart should be studied and shifts made in operations, so that the minimum crew may be used. Sample charts are presented. W. H. Martin

537. Apparatus for treatment of fluids requiring sterilization or pasteurization. W. H. COULTER. U. S. Patent 2,799,216. 5 claims. July 16, 1957. Offic. Gaz. U. S. Pat. Office, **720**, 3:497. 1957.

A continuous high-temperature, short-time pasteurizer or sterilizer, in which rapid heating is provided by passing an AC current between two electrodes located in the stream of a heat-sensitive, fluid-like milk. An accurate holding period is provided, followed by a cooling zone. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

538. Formal funded pension plans. P. V. OSBORNE, Towers, Perrin, Forster & Crosby, Inc., Philadelphia, Pa. S. Dairy Prod. J., **61**: 2:128. 1957.

An adequate formal funded pension plan increases efficiency of an organization, allows advancement of younger men, and helps to attract competent personnel. Uniform treatment of employees helps to avoid management problems. A formal funded plan recognizes human depreciation as a charge against current operating expense during the employee's productive years, instead of deferring it as a liability against future profits. Employer contributions for this purpose are deductible from taxable income. Employees may or may not contribute. Plans and costs may be varied to fit the needs of each organization. F. W. Bennett

539. Restaurant promotions and how they can be used to boost dairy product sales. W. K. HOLM, Ind. Dairy Prod. Assn., Indianapolis. S. Dairy Prod. J., **61**, 2:29. 1957.

Restaurant operators are interested primarily in increasing total sales. The "Milk Now and Coffee Later" slogan and the old silver dollar idea have been very successful promotions. Furnishing and helping set up display materials, operating booths at restaurant shows, and offering to help train personnel have been aids in promoting good will. F. W. Bennett

540. Food store promotions. P. R. OLIVER, Foremost Dairies, Inc., San Francisco, Calif. S. Dairy Prod. J., **61**, 2:28. 1957.

When the grocer recognizes that dairy products represent 9% of total store sales, are first in producing store traffic, compare favorably with other items in sales and profits per square foot, and rank high in turnover, he is ready for planned store promotion.

A format for promotion should be based upon the facts that a young mother's interest is centered in the family, time is an important factor, she is an impulse buyer, and is budget-minded. Promotions should be scheduled far enough in advance that they may be included as part of the store's merchandising calendar. Without discontinuing the promotion of whole milk as Nature's most nearly perfect food, public interest provides an excellent opportunity for the promotion of low-calorie dairy products. F. W. Bennett

541. Coordinating the efforts of your production and sales departments. W. R. KISER, White Ice Cream and Milk Co., Wilmington, N. C. S. Dairy Prod. J., **61**, 1:24. 1957.

An annual plan of operations of the business, based upon sales and production potentials of the plant, is recommended. This should be the basis of quarterly, monthly, weekly, and daily planning, with adjustments being made as may be necessary for each period. Close communication between production and sales forces, and enthusiasm, are essential to efficient growth of the industry. F. W. Bennett

542. Simplified system of route accounting. R. P. KAPP, Edisto Farms Dairy, Columbia, S. C. S. Dairy Prod. J., **61**, 1:21. 1957.

The route accounting system of Edisto Farms Dairy for milk and ice cream is described. All

retail sales are for tokens and cash or on monthly charge accounts. The office force is divided into cashiers, sales office, and general office. Each is responsible for certain duties. Deliveries, charges, and receipts are controlled by individual routes. Route salesmen compute statements, which are verified by supervisors and office personnel. Statements are microfilmed. The route salesmen are checked in full daily, and are responsible for shortages at the end of the month. Returned checks are charged back to the routes to which they apply. A Multilith machine is used for printing forms and letters. A folding machine, postage meter, rapid letter-opener, and bookkeeping machine save time in the office. F. W. Bennett

543. Making full use of your accounting system. L. L. HYKE, Coburg Dairy, Inc., Charleston, S. C. S. Dairy Prod. J., **61**, 1:21. 1957.

Details of procedures in handling records of different types by machine are described. Machine accounting affords advantages in speed, accuracy, elimination of peak loads of work, easier detection of errors, neatness and simplicity of the work, resulting in greater legibility and ease of correction. F. W. Bennett

544. Gadgets, forms, and methods designed to reduce detail of office labor. M. G. HUGO, Dairy Accounting Serv., Charlotte, N. C. S. Dairy Prod. J., **61**, 1:20. 1957.

Some suggestions for improving office efficiency are the use of forms with same listing of items, elimination of duplication, microfilming statements, elimination of daily posting of tickets, the use of labor-saving office equipment, employees suited to their respective jobs, conveniently located files and equipment, elimination of unnecessary reports, and arrangement of desks in such a way as to avoid excessive conversation. F. W. Bennett

545. Maintaining temperatures in refrigerated delivery trucks. R. F. GUILFOY, JR., Transportation and Facilities Branch, Agr. Mkt. Serv., USDA. Ice Cream Rev., **41**, 2:26. 1957.

The use of plastic curtains inside the doors of refrigerated trucks has proved helpful in maintaining the temperature of the frozen products, according to results of a study conducted by the USDA. When the curtains were used, the products averaged 3.2° F. colder, and air temperature inside the truck 7.2° F. lower than when similar trucks were used without the curtains.

The use of the curtains virtually stopped the dripping of moisture from the cold hold-over plates, and eliminated the necessity of carrying dry ice in the trucks as supplemental refrigeration.

It is believed that plastic curtains could be used to good advantage on trucks used in the local delivery of milk and ice cream.

W. J. Caulfield

546. Butterfat and volume losses. M. W. BOND, H. P. Hood & Sons, Boston, Mass. S. Dairy Prod. J., **61**, 2:150. 1957.

Control of losses should be based upon locating the particular operation at fault, making the responsibilities for operations clear and distinct to the individual operators, providing tools of measurement which are well within the tolerance of expected results, instituting the necessary personnel training and practices, checking each operation early enough to allow for corrections, and making office reports to management accurately and promptly.

F. W. Bennett

HERD MANAGEMENT

547. Pipeline-milker and bulk-tank milk filtration. J. M. JENSEN AND L. JOKERY. Dept. of Dairying, Mich. State Univ., East Lansing. J. Milk and Food Technol., **20**, 230. 1957.

Bonded nonwoven cotton discs showed greater filtration efficiency than flannel or muslin fabric. Greatest uniformity in numbers of particles was secured when testing 1-gal. portions from bulk tank milk. Bag filters of flannel and muslin fabric resulted in inadequate filtration.

H. H. Weiser

548. Milking apparatus. N. CORDIS. U. S. Patent 24,337. 3 claims. July 16, 1957. Offic. Gaz. U. S. Pat. Office, **720**, 3:447. 1957.

Essentially the same as Abstract No. 604, Journal Dairy Science (Abstracts), **33**: A120. 1950. R. Whitaker

ICE CREAM

549. Ice cream packaging for self-service merchandising. V. S. PACKARD, E. L. THOMAS, AND W. B. COMBS, Univ. of Minn., St. Paul. S. Dairy Prod. J., **61**, 2:22. 1957.

The highest and most widely fluctuating temperatures were found usually at the top level of self-service ice cream cabinets. Body and texture changes and shrinkage were pronounced during from 4 to 5 wk. of storage at top level in waxed fiber, polyethylene, and styrene containers, but aluminum foil overwraps, exterior foil laminates, and solid aluminum containers effectively retarded body and texture changes and prevented shrinkage. A foil laminate liner in waxed fiber containers was ineffective. Colored lacquers did not affect the protection by aluminum. The protective ability of foil appeared to be due primarily to its heat-conductive capacity and, to a lesser degree, to its radiant heat-reflective ability. A heavy insulated "Jiffy" bag, and a corrugated cardboard sleeve with a foil overwrap, were equal in insulating ability. F. W. Bennett

550. Factors affecting production rate, quality of product, and freezer performance in making ice cream. H. L. MITTEN, JR., Creamery Pkg. Mfg. Co., Chicago, Ill. S. Dairy Prod. J., **61**, 1:30. 1957.

There appears to be a trend toward the production of extruded products for which a stiff ice cream usually is required. The ice cream mix is as important as the freezer in producing a satisfactory product. The freezers being manufactured today are designed to meet the trend. Undersized or long ice cream lines may cause breakdown of the body and texture of the ice cream from excessive pressures. Skilled technical personnel is more important today than ever before. F. W. Bennett

551. Pure vanilla best for quality. W. S. Steinitz, American Food Labs., Inc., Brooklyn, New York. Ice Cream Rev., **41**, 2:27. 1957.

Pure vanilla can enhance and upgrade the flavor quality of ice cream only when the mix is free of undesirable flavors and when an adequate amount of flavor is used. Off-flavors from ingredients, flavors developing during storage, or flavors resulting from faulty processing will mask the delicate flavor of pure vanilla.

The amount of pure vanilla required to produce a given intensity of flavor will vary with the composition of the mix. As the fat and total solids of the mix are increased, the quantity of pure vanilla used should also be increased. Whereas from 4 to 5 oz. of pure vanilla may be adequate per 10 gal. of ice cream using a mix with 37% total solids, from 5 to 6 oz. would be required to produce the same flavor intensity using a mix containing 40% total solids.

The full benefits of using pure vanilla can be achieved only when the mix is compounded from fine-flavored ingredients, and when an adequate amount of flavor is used.

W. J. Caulfield

552. Production, handling innovations. E. Thom. Ice Cream Review, **41**, 2:28. 1957.

Automatic packaging of a tapered ½-gal. carton, the use of a measuring meter for liquid dairy ingredients, and the use of trucks with a walk-in door at the rear are three innovations introduced by the Northland Milk and Ice Cream Co. of Minneapolis, Minn.

Automatic filling of the tapered ½-gal. carton equipped with handles on top has made it possible to package 800 gal. of ice cream per hr. with a crew of three persons. Prior to the introduction of automatic filling, a crew of seven persons was required for this same operation.

The use of a new sanitary measuring meter, accurate to ½ of 1%, for the liquid dairy ingredients has eliminated the weighing operation, and is providing management with a closer check on the purchase and utilization of these ingredients than it has ever had before. Installation of another meter is planned to check mix which is purchased by the company.

The use of delivery trucks equipped with a walk-in door at the rear speeds up the loading operation and facilitates the easy arrangement of stock for delivery to the customers.

W. J. Caulfield

553. Frozen confection. S. Oprean. U. S. Patent 2,801,922. 9 claims. Aug. 6, 1957. Offic. Gaz. U. S. Pat. Office, **721**, 1:134. 1957.

A coated item consisting of a portion of frozen confection surrounded by a foamy layer of emulsified gelatinous material.

R. Whitaker

554. Ice cream container. M. J. Kelly (assignor to Paper Products Co.). U. S. Patent 2,800,266. 1 claim. July 23, 1957. Offic. Gaz. U. S. Pat. Office, **720**, 4:777. 1957.

A tubular-shaped container for ice cream, with a tear strip, is described. R. Whitaker

MILK AND CREAM

555. Beverage container. W. A. Pugh. U. S. Patent 2,799,439. 4 claims. July 16, 1957. Offic. Gaz. U. S. Pat. Office, **720**, 3:560. 1957.

A paper container having a gable-type top and equipped with a paper hanger beneath a covered opening in the gable, holding a sipper straw which may be withdrawn to provide means of sipping the contents. R. Whitaker

556. Carrier construction. R. F. Gegan (assignor to Pontiac Engraving and Electrotype Co.). U. S. Patent 2,801,129. 8 claims. July 30, 1957. Offic. Gaz. U. S. Pat. Office, **720**, 5:1007. 1957.

A device for attaching two gable-top cartons together and to facilitate carrying.

R. Whitaker

557. Leak-proof container. C. E. Kruszynski. U. S. Patent 2,801,041. 2 claims. July 30, 1957. Offic. Gaz. U. S. Pat. Office, **720**, 5:984. 1957.

A paper container of the gable-top type is described, with a leak-proof inner liner of pliable material which is closed by crimping with the outside paper along the top ridge.

R. Whitaker

558. Refrigerated cream and butter dispenser. I. O. Meltesen. U. S. Patent 2,801,529. 4 claims. Aug. 6, 1957. Offic. Gaz. U. S. Pat. Office, **721**, 1:35. 1957.

A dispenser suitable for restaurant use.

R. Whitaker

559. Apparatus for whipping cream and the like. P. W. Malloy (assignor to W. M. Malloy). U. S. Patent 2,800,311. 2 claims. July 23, 1957. Offic. Gaz. U. S. Pat. Office, **720**, 4:789. 1957.

A motor-driven cream whipper is described, having a device which deflects the surface layers of the product downward toward the beater.

R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

560. Protein supply for human nutrition. C. G. King, Nutrition Foundation, Inc., New York. J. Agr. Food Chem., **5**: 340. 1957.

Protein malnutrition, often the No. 1 health problem in underdeveloped areas, is discussed.
S. Patton

PHYSIOLOGY AND ENDOCRINOLOGY

561. Metabolism of insecticides. Metabolism and excretion of phosphorus-32-labeled diazinon in a cow. W. E. Robbins, T. L. Hopkins, and G. W. Eddy, Entomol. Research Branch, Agr. Research Serv., USDA, Corvallis, Ore. J. Agr. Food Chem., **5**: 509. 1957.

Phosphorus-32-labeled diazinon, administered orally to a cow at 20 mg. per kg., is rapidly metabolized and excreted. Only low levels of unchanged toxicant were found in blood and milk samples. About 74% of the dose, excreted as polar degradation products, was accounted for in the urine 36 hr. after treatment.
S. Patton

562. Physiological aspects of fluids in female genitalia, with special reference to cattle—A review. D. Olds and N. L. VanDemark, Univ. of Ill., Urbana. Am. J. Vet. Research, **18**, 68: 587. 1957.

A brief, concise review with 336 references is presented. Major discussion sections are, *(1)* endocrine control of cellular activity, *(2)* histological and histochemical features, *(3)* characteristics of luminal fluids, and *(4)* significance of luminal fluids. It is emphasized that the proper luminal ffuids are essential for sperm transport and for viability, fertilization, and resistance to infections. E. W. Swanson

563. Utilization of carbohydrates by the young calf. A. M. Dollar and J. W. G. Porter. Nature, **179**, 4573: 1299. 1957.

For the preruminant calf only glucose and lactose are suitable carbohydrates. Once the rumen flora is established, starch becomes suitable because of the action of the bacterial enzymes. R. Whitaker

564. The behavior of spermatozoa in luminal fluids of bovine female genitalia. D. Olds and N. L. VanDemark, Univ. of Ill., Urbana. Am. J. Vet. Research, **18**, 68: 603. 1957.

Fluids were collected from the oviducts, follicles, uteri, and vaginas of 16 cows in different known estrual stages, and from 18 other cows. The motility and viability of bull sperm were determined in each fluid. The utilization of glucose and oxygen, and the production of lactic acid by sperm, in each fluid diluted with saline, were also measured. Motile sperm were observed in fluids of follicles, oviducts, vaginas, and uteri, respectively, for averages of 19, 12, 9, and 7 hr. Sperm metabolic activity was in the same order of fluids as motility. Uterine fluid and cervicovaginal mucus inhibited metabolic activity, but oviduct fluid and follicular fluid enhanced it over saline controls. Sperm in follicular fluid were frequently agglutinated, but their tails remained motile. Hyaluronidase and phosphate buffer slightly increased sperm survival in mucus. E. W. Swanson

SANITATION AND CLEANSING

565. Milk pipe-line washing system. T. W. Merritt (assignor to Babson Bros. Co.). U. S. Patent 2,800,134. 6 claims. July 23, 1957. Offic. Gaz. U. S. Patent Office, **720**, 4: 742. 1957.

A method of cleaning a pipe-line milker by recirculation of cleansing and rinsing solutions, and a means of draining the cleaned pipe-line.
R. Whitaker

Where Nestlé's Lab Work Ends

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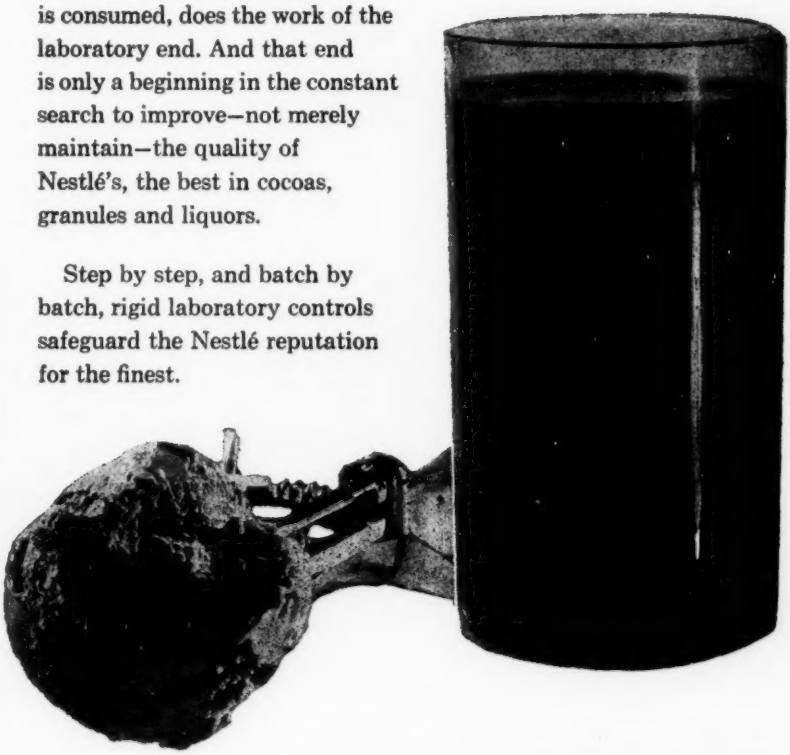

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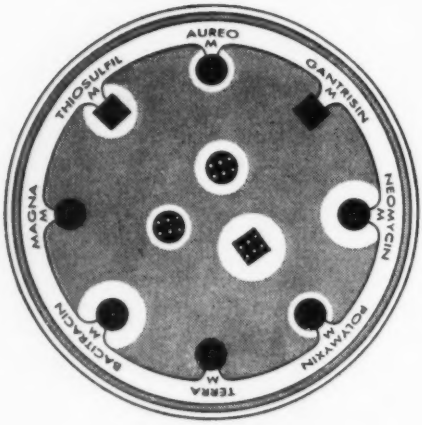

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